CROONIAN LECTURE.—" The Cosmical Function of the Green Plant." By C. TIMIRIAZEFF, Professor of Botany in the University of Moscow. Communicated by Sir M. FOSTER, Sec. R.S. Received April 30,—Lecture delivered April 30, 1903.

[PLATES 20—22.]

The first object which attracted Gulliver's notice when on his visit to the Academy of Lagado was a man of " meagre appearance," his eyes fixed on a cucumber sealed in a phial. On Gulliver's questioning him, the strange personage explained that for more than 8 years he had been absorbed in the contemplation of this bit of apparatus, trying in vain to solve the problem of the storage of the sun's rays in this recipient and their possible utilisation.

Now, to begin with, I must frankly confess that I am just that sort of man : for 35 years have I been staring, if not exactly at a cucumber in a phial, still at what comes to the same thing, at a green leaf in a glass tube, and breaking my head in vain endeavours to clear up the mystery of " bottled sunshine." If I venture to bring before this illustrious Society the modest results of this long-continued work—it is in the hope that this theme may have a real, though very distant connection with the subject which Dr. Croon, the generous and enlightened founder of this lectureship, considered as most fit for the occasion. During a long series of years the chief topic of these lectures was " Muscular Motion," and at a more recent period " Motion in Animals and Plants," and " The Origin of Vital Movements " in general. Perhaps I may be allowed to take a step further in this direction—in fact, the last possible step, and speak of the energy manifested in all these movements, of its remotest source—the sunbeam stored in the green plant.

I suppose it is hardly necessary to remind you that the ground we are going to tread has been explored in this country and America for more than a century. Suffice it to call to mind the familiar names of Priestley, Count Rumford, Daubeny, Sir David Brewster, John William Draper, Sir John Herschel, Robert Hunt, Sir George Gabriel Stokes, Mr. Edward Schunck, Mr. Sorby, Sir William Abney, Mr. Blackman, and last—not least—Mr. Horace Brown, to show what interest this question has continued to inspire under its various aspects. I may add that it was in Professor Tyndall's brilliant " Heat a Mode of Motion " and in my resultant study of Robert Mayer's classical work " Die organische Bewegung in ihrem Zusammenhange mit dem Stoffwechsel," that I found the first impulse towards all my subsequent work. And it must be admitted that the moment was singularly propitious :

Bunsen and Kirchhoff had just discovered that powerful means of research—spectrum analysis; Sir George Stokes had applied it to the study of the colouring matter of blood; Desains and Tyndall had worked out Melloni's thermoscopic methods; Henri Sainte-Claire Deville had made his great discovery of the dissociation of carbon dioxide; Bunsen, by simplifying the methods of gas analysis, had quite recently put them at the disposal of the physiologist, and lastly, Boussingault had just published his classical researches on the assimilation of carbon, showing that this process could be easily studied on leaves or even on pieces of leaves detached from the living plant.

From the very outset, on the first page of my first Russian paper, which appeared in 1868, I formulated the problem in all its generality in the following lines: "To study the chemical as well as the physical conditions of this process, to follow the solar ray that effects it directly or indirectly, up to the moment when we see it vanish on being trans-formed into internal work, to find out the quantitative relation between the energy absorbed and the work done—here lies the brilliant though perhaps arduous problem in attacking which modern physiologists ought to unite all their forces."

And I may now add that the very moderate results obtained after a long series of years only confirm that, at all events, I did not overrate the difficulties of the problem.

When I first set to work, the current idea was that the photo-chemical process going on in the green leaf under the influence of light ought to be considered a function of its luminosity. This belief was chiefly based on J. W. Draper's classical experiments on the spectrum. From a theoretical point of view it seemed to me highly improbable that a chemical process so essentially endothermic, and consequently depending on energy of radiation, should stand in a direct relation to a purely physiological property of radiation, having no existence outside the organ of sight. Light, taken in the narrow acceptance of the word, does not exist for the vegetable world. But facts brought forward by such an authority in this line of research as Draper were not to be so easily dismissed on the single ground of their improbability. However, a careful study of Melloni's classical memoir on the shifting of the maximum of heat with the state of purity of the spectrum, brought me on the track of a considerable experimental flaw in Draper's researches. His spectrum was highly impure; in fact it was obtained by means of a circular aperture $\frac{3}{4}$ of an inch in diameter—Wollaston's narrow slit not seeming to be in general use at the time. A sufficient explanation was thus obtained for the coin-cidence of the chemical effect with the maximum luminosity in the yellow and green rays, this part of the spectrum being practically white, slightly tinged with these colours, and consequently acting by

the sum of nearly all the rays of the spectrum, its limits only remaining monochromatic.

The next step was to prove that this coincidence of the two maxima of luminosity and of the chemical effect did not in reality exist. A simple inspection of these three curves (the curve of assimilation, that of luminosity and the energy curve), taken from my first (and last) German paper, proves with sufficient evidence that a coincidence of the chemical effect with Fraunhofer's luminosity curve was out of the question; but, so far as the visible spectrum is concerned, there exists a decided relation with the energy curve.

In this first and preliminary research the more simple and convenient method of coloured liquid screens, introduced by Senebier and applied in a more exact form by Daubeny, was adopted. I merely introduced a more correct way of calculation and graphical representation of the results obtained. At the same time I applied the method of gas analysis now in general use, though quite erroneously attributed to Professor Pfeffer.

But if these results were sufficient to dispose of the current ideas on the importance of the luminosity, based as they were on Draper's experiments, they were not sufficient for the building of another theory. It was impossible, for instance, to consider the decomposition of the carbon dioxide as simply an effect of the relative energy of the radiation. The maximum of energy in a prismatic spectrum lies in the infra-red, and a remarkable experiment of Cailletet (curiously too often omitted in most of the historical sketches of the subject) gave an unequivocal proof that the rays of light, filtered through Tyndall's solution of iodine in carbon bisulphide, were incapable of producing the reduction of the carbon dioxide in a green leaf.

It was evident that some other principle had to be brought forward in order to explain the absence of any chemical action in the invisible part of the spectrum, as well as its distribution in the visible. The principle that was appealed to was Sir John Herschel's law: that a photo-chemical reaction may be induced by those rays only that are absorbed by the substance undergoing a change and consequently, as a rule, presenting a complementary colour. It was supposed that, applied to our case, Herschel's law would mean that the reduction of the carbon dioxide must take place at the expense of those rays of the spectrum which are absorbed by the green matter of the leaf—that they must correspond to the absorption bands of chlorophyll.

Lommel is generally credited with having been the first to enunciate the idea that the reduction of the carbon dioxide may be considered as a function of the energy of radiation and of the degree of its absorption by chlorophyll. But this opinion, despite its being very general, is none the less erroneous. Lommel himself admits that the first half of the proposition, concerning the dependence on the energy

of radiation, was formulated by me, and I may add that I could not affirm the second half of the truth for the simple reason that it had already been affirmed before me, and consequently before Lommel, by Jamin and Edmond Becquerel. It is only a matter for wonder that, as a physicist, Lommel was not acquainted with Jamin's well-known text-book and the perhaps still better known book of Becquerel " La Lumière, ses Causes et ses Effets."

But of course the main thing was not to express an idea, however exact, but to furnish an experimental proof of its exactness. *Nullius in verba* is now, as it was centuries ago, the watchword of every man of science. In other words, it was necessary to repeat Draper's celebrated experiment without falling into his error, and there was the chief difficulty. It is generally admitted that this proof was furnished for the first time by my old friend Professor N. Müller, but I am sorry to say that on this point I am again obliged to contradict the general opinion. In fact Müller never furnished the direct proof of this connection between the chemical process and the absorption of light, and he could not do so because he did not possess the only means of escaping Draper's error. In his first work, previous to my researches, he experimented in a tolerably pure spectrum, but the light intensity was then insufficient and he could not actually obtain any reduction of the carbon dioxide, but only inferred that it took place, judging by the differences in the intensity of the respiration. In his second paper, which appeared after my publication, he could affirm directly the reduction of carbon dioxide, but only at the cost of the purity of his spectrum. In fact the width of his slit was nearly the same as in Draper's experiment, and the result was practically the same; this time he obtained the maximum effect in the yellow rays.

If I allow myself to insist on this point somewhat in detail it is not from any vain desire to establish my priority, but because it is my firm conviction that the method adopted by me is now, as it was at that time, the only means of avoiding Draper's error. Müller did not devise any means of avoiding and consequently had no chance of escaping it. The dilemma, as fully exemplified by Müller's failure, was the following : if the spectrum is pure the intensity of light is not sufficient to obtain a reduction of carbon dioxide ; if, on the contrary, we open the slit in order to increase the intensity of the spectrum, we may be sure beforehand of obtaining the maximum effect in its middle, somewhere in the yellow or green rays. There was but one means of escaping the two horns of this dilemma—it was to increase the intensity by diminishing the dimensions of the spectrum ; but then the leaf surfaces being reduced in proportion, the quantities of gas to be analysed would be too small to be measured in such gasometric apparatus as the chemist of the time could put at the disposal of the botanist.

The main object was to alter, so to speak, the scale of the gasometric measurements without altering the precision of the method and the convenience of all the operations. Though I have often since that time changed the details, the principle of the method I adopted remains the same. The leaves are introduced into cylindrical tubes of suitable diameter, or still better into flat tubes in order to increase the

Fig. 1.
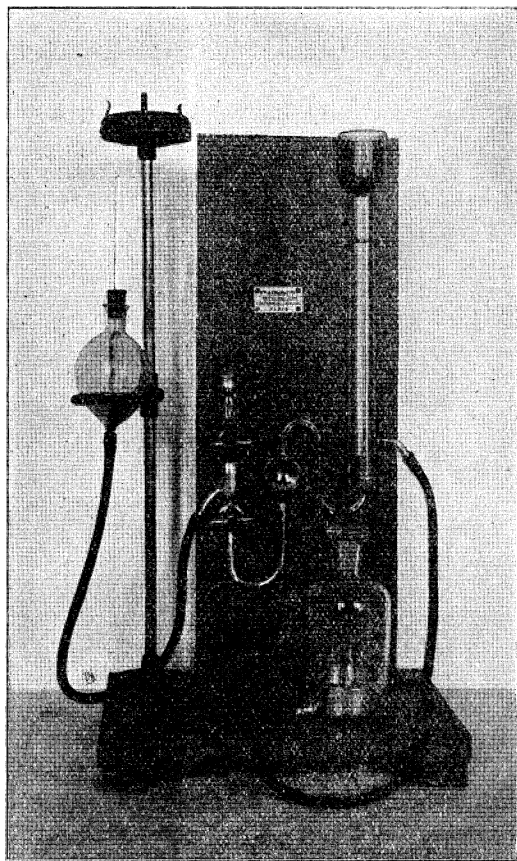
green surface, and the gases are measured and analysed in a tube of the smallest possible bore. All the necessary manipulations, the transfusion of gases as well as the treatment by liquid re-agents, are easily performed by using this form of gas pipette, which I introduced in 1871, and still (fig. 1) consider the most convenient. The gas pipette and the mercury trough are rigidly connected, allowing us to give to the ascending branch of the syphon an outer diameter not exceeding

1·5 mm., so that it may be easily introduced into eudiometers of 2 mm. inner diameter.   This method has also the advantage that no stopcock or rubber is used.

All the necessary manipulations may be thus briefly summed up ; thanks to this modified form of Bunsen's gasometer, the necessary gas mixture on its way through this burette is, so to speak, cut into slices of suitable volume and distributed to the leaves in the flat tubes.   After the necessary exposure to the spectrum the gases are extracted by means of the pipette just described, and introduced for analysis into these eudiometric tubes, which easily allow the estimation of 1/1000 of a cubic centimetre.

Thanks to all these contrivances, the problem of obtaining trust-worthy analytical data with green surfaces exposed to a pure spectrum was for the first time solved, and I still consider this method the only one that may be safely relied upon in the study of this question.   The two other methods that have been proposed are far less exact.   Count-ing the number of gas bubbles emitted by small aquatic plants, which may be demonstrated as a pretty lecture-room experiment, even if their diameters are measured under the microscope, as recently proposed by Kohl, is hardly to be recommended.   It is true, we may obtain much better results when using what I call my *microeudiometer*—a small piece of apparatus which permits us to measure and analyse in a couple of minutes a bubble not bigger than a pin's head.   Though reduced here to a minimum, the sources of error inherent in the aquatic nature of the plant are still not fully eliminated.

As to the third method, Engelmann's well-known bacterium method, I still consider that it is of rather too indirect a character, and persist in my opinion, expressed many years ago, that chemical pro-blems ought to be studied by chemical means.   Moreover, we shall presently see how little this method is to be relied upon.

Thus we see that the introduction of a gasometrical method allowing the measurement and analysis of very small quantities of gas made it for the first time possible to study the assimilation of carbon in a pure spectrum.*

There were two more points to be considered before handling this problem of the connection between the photo-chemical process and the absorption of light in the green leaf.   The spectra of chlorophyll and

* In order to illustrate the difficulties I had to contend with, I may quote one of the best authorities in this line of research, M. G. Lemoine.   Speaking at so recent a date as 1895 (' Annales de Chimie et de Physique,' Ser. 7, vol. 6, December) of his experiments on the decomposition of oxalic acid in sunlight, M. Lemoine says: " Les expériences devraient être faites, non seulement avec la lumière blanche, mais encore avec ses différentes radiations.   En pratique, il est impossible de les isoler complètement, et si on le pouvait, *on n'aurait plus assez d'intensité pour des mesures quantitatives.*"   My method may be thus summarised : a heliostat with the largest mirror and a gas burette of the smallest bore.

of its nearest products had to be studied more thoroughly than they had been. It would lead me too far out of the way if I were to give an account of my researches published in a small Russian volume in 1871. My plates represent an attempt at a classification of chloro-phyll and its products based on their spectroscopic characters, an attempt that seems to me fully confirmed by the more recent and detailed researches, especially of the late Mr. Edward Schunck, of Marchlevsky, and of Professor Hartley. I will pause for one moment on the method of representing the spectra which I adopted at the time and have since been trying to improve. Till then the spectrum was generally represented as consisting of distinct black bands separated by bright intervals, and it corresponded to a certain more or less arbitrary state of concentration or thickness of layer. Plotting together whole series of spectra corresponding to layers of varying thickness, I obtained the following spectrograms. At a later period I substituted, for a somewhat troublesome method, the direct inspection of the solutions in wedge-shaped troughs—a method, if I am not mistaken, introduced by the late Professor Gladstone—and lastly contrived to obtain directly these spectrophotograms. It is a curious fact, to be mentioned *en passant*, that so far as I know photography has till now been applied only to the more refrangible and not to the less refrangible part of the spectrum of chlorophyll, which is of the highest interest to the botanist.

Here are some photographs that I obtained, in 1892 (fig. 2), on ordinary Ilford plates, which were also used for the first spectrophoto-

FIG. 2.
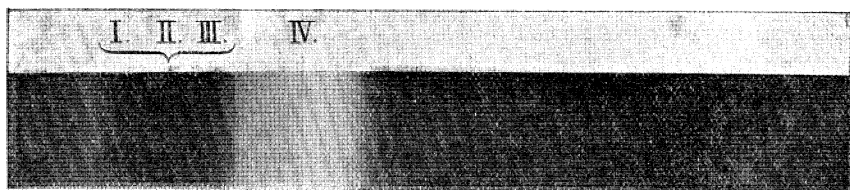
Photographic Spectrum of Chlorophyll.
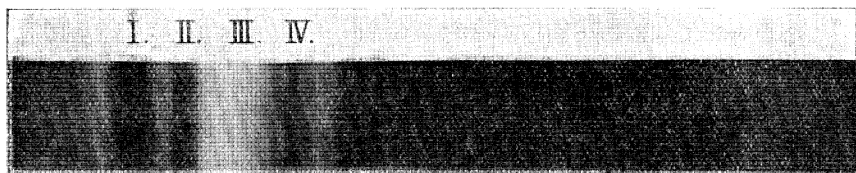
Modified Chlorophyll (Stokes), same Concentration.

grams of 1895, but now I prefer Cadett's excellent spectrum plates. The most convenient form of this wedge-shaped cell for photographic use is the following (fig. 3, A). A cubical glass cell is divided by a glass partition into two prismatic compartments, one of which is filled with the coloured solution, the other with the solvent. A horizontal slit is, on the whole, preferable, since it permits us to alter easily the concentration of the solution so as to obtain the desired spectrogram, and the swing-back of the camera may be used for focussing the spectrum. In these photo-spectrograms (fig. 3, B) we have, of course, the most convenient way of representing absorption spectra, but we shall see later that in certain cases we shall still be obliged to have recourse to another method—the spectrophotometric.

Returning now to our chief problem, we may see how important it was to know the real form of the absorption curve. All the spectra generally represented being discontinuous, showing sharp bands alternating with light intervals, it was rather puzzling to account for the continuous effect, as for instance, in Draper's experiments.

Armed with the necessary gasometric and spectroscopic methods of research, I could at last repeat Draper's classical experiment without the fear of repeating his error. The result of my experiments was a strict confirmation of the applicability of Sir John Herschel's law to our case. Not only do the two maxima coincide (fig. 4), but the assimilation curve reproduces very nearly the absorption curve, so far at least as the less refrangible part of the spectrum is concerned. It is to be remarked that the secondary maxima of absorption seem to have no effect, but it is subject to doubt, especially after the latest researches of Schunck and Marchlevsky, whether they appertain to the principal chlorophyll constituent or to some products of its decomposition.

The same results were obtained later by quite another method. The dissociation of carbon dioxide represented by this curve (fig. 4), the "photolysis," as it may be called, is the first and by far the most important stage of the whole process, being directly dependent on the external source of energy. But it is closely followed by the photosynthesis of organic matter. Though, thanks to the brilliant researches of Horace Brown and Morris, we now know that starch is neither the first nor by far the only product of this synthesis, still it is a product that follows the reduction of the carbon dioxide at the short interval of some minutes, and, what is of still greater importance, its presence may be easily shown by the well-known iodine test.

If the reduction of carbon dioxide be considered a function of those waves of light which correspond to the chlorophyll absorption bands, and, on the other hand, the production of starch is the next stage of the same photo-chemical process, it may be fairly presumed that this production of starch in a spectrum will be strictly localised, restricted
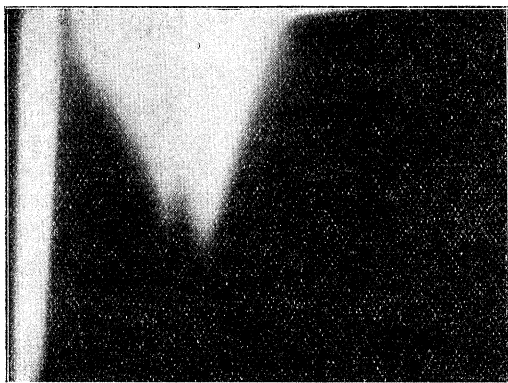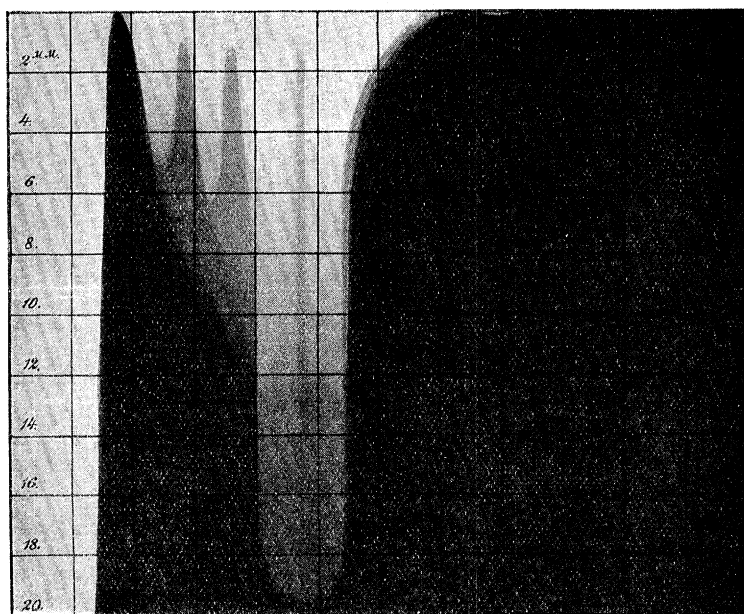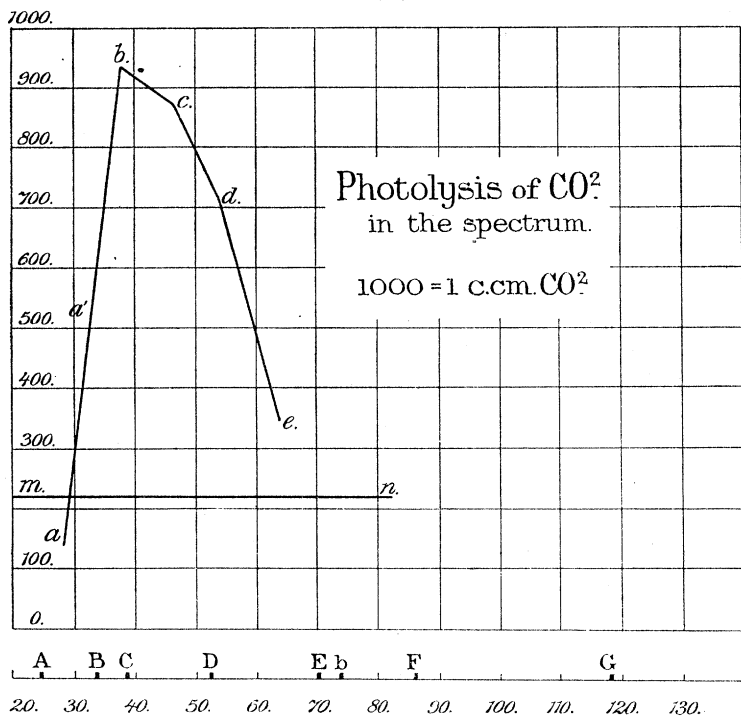
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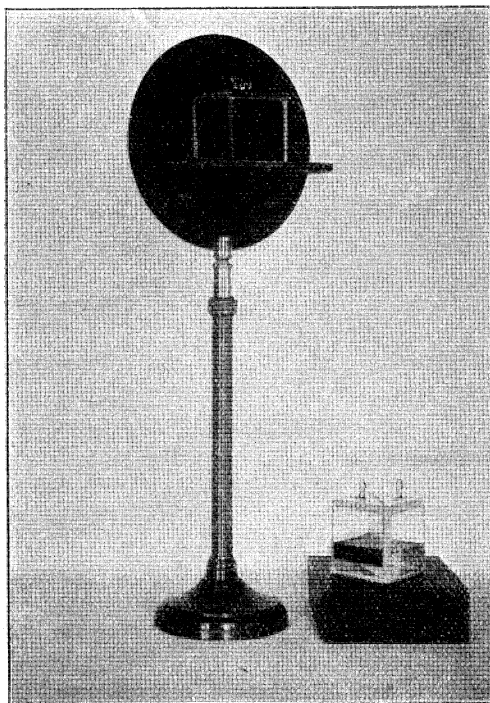
A.

B.

Apparatus for taking Photographs and the direct Projection of Spectrograms with the Lantern.

Fig. 4.



Photolysis of CO_2
in the spectrum.

$1000 = 1$ c.cm. CO_2

to the absorption bands of chlorophyll.   In other words, a spectrum of
sufficient intensity, projected on a living leaf previously depleted of its
starch, will impress in this leaf an invisible image of the chlorophyll
absorption spectrum formed of starch grains.   This latent image may
be developed by the iodine test.

These previsions have been fully realised in the following experiment.
Healthy leaves attached to the living plant (a Hydrangea) but
previously deprived of their starch, after an exposure of 5—6 hours to
the influence of a small but very bright and pure spectrum, were
treated in the well-known way with an iodine solution.   They
exhibited a well-defined spectrum of chlorophyll with its principal band
between B and C very prominent, and a gradual falling off towards
the blue end (fig. 5).   Just as in the case of the reduction curve, no
secondary bands could be detected.

Fig. 5.
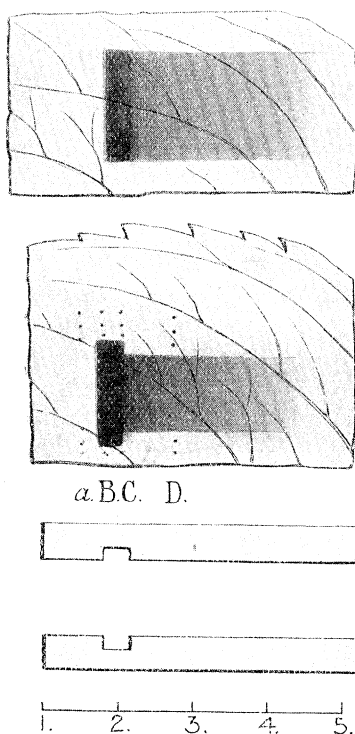
Photosynthesis of $C_6H_{10}O_5$ in the Spectrum.

The blue and violet rays produced hardly any effect.   This last
result may be chiefly attributed to the fact that the appearance of

starch is only the resultant of two conflicting processes, of its production and its dissolution, so that at a certain point when the assimilation is not sufficiently intense the former may be counterbalanced by the latter, no surplus starch being stored in the chloroplast.

This leads us to the discussion of the very important question concerning the relative effect of the rays of the more refracted part of the spectrum.   It might have been remarked that my researches were restricted to its less refracted half.

Concerning the other half, the current opinion was that these rays play a comparatively small part in our photo-chemical process. This opinion was considered the result of Draper's classical experiments and of a great many experiments with yellow and blue coloured screens.   But all these latter experiments are little to be relied upon, since it may be proved by photo-metrical measurements that the intensity of the transmitted light is diminished in very different proportions by these media, and that in consequence the results obtained are not directly comparable.   And they are likewise unreliable when the prismatic spectrum is used, because of the great dispersion of these rays, and that was the reason why I confined myself to the less refrangible half of the spectrum and did not push the experiment further into the blue and violet rays.   It may be shown, by-the-bye, how capricious and unreliable in this respect are the results obtained by Engelmann's bacterium method.   It will suffice to put *en regard* the results obtained by Engelmann and those of one of his most faithful adherents—Professor Pfeffer.   In the former case we have a distinct second maximum corresponding to the blue rays—in the latter no such maximum could be observed.

It might seem that the defect inherent in the spectrum method might be easily remedied by a simple calculation, assuming that the effect is proportional to the intensity.   But the law which regulates the relation of the process to the intensity of light not being known, this assumption would be gratuitous, and in fact we shall see later that this law is much more complicated.

The simplest experimental way of solving the question would, of course, seem to be to make the experiment in a diffraction spectrum. But a series of experiments I made with a beautiful Rowland grating gave only a negative result—just as in Müller's first experiments—the light intensity not being sufficient, no reduction of the carbon dioxide could be directly revealed.   In consequence, I was obliged to fall back on the prismatic spectrum.   I may, perhaps, be allowed to refer to the fact that even Mr. Langley, notwithstanding the wonderful sensitiveness of his bolometer, remarks in one of his latest papers, " The prism is, on the whole, far more convenient than the grating."

To work with the prism, care being taken to avoid the error arising from the differences of dispersion, was in this case simply to adopt the

well-known method used by physicists for recomposing the prismatic spectrum. We may thus combine the intensity of the prismatic spectrum with the advantages of the diffraction spectrum, the gasometric results being directly comparable. I used the very ingenious apparatus devised by the late Jules Dubosq for the production of complementary colours. It consists of a cylindrical lens and of a wedge-prism fixed on a plate of glass. Instead of a spectrum we obtain two stripes of light of equal area and of complementary colours.

More recently I proposed a modification of this apparatus (skilfully executed by M. Pellin) consisting of two glass plates with their respective prisms, which may be made to slide past one another and divide the spectrum into three parts (see Plate 20, A, b).

The glass tubes containing the leaves being immersed in mercury (Plate 20, A, a), the spectrum must of course be divided in the vertical sense, but in other cases it may be more convenient, as will be presently seen, to adopt Kundt's method of dividing it in the horizontal sense so that the two coloured surfaces may be put into direct contact (Plate 20, B, c). As I have just said, this method combines the advantages of the grating and of the prism, the difference of dispersion being eliminated as in the diffraction spectrum, the intensity remaining the same as in the prismatic.

Moreover, in this last respect the method may present even an advantage over the simple prismatic spectrum. If desirable the spectrum used may be in a considerable degree impure (that is, obtained by means of a broad slit), provided the stripes of light to which the leaves are exposed are of a homogeneous colour and present equal areas. We have but to place behind them a small Sorby-Browning spectroscope (Plate 20, B, a) with a scale giving the wavelengths, and notwithstanding a certain degree of overlapping of the portions of the spectrum corresponding to the coloured stripes, the analytical results may be directly plotted on a normal spectrum scale. Thus, thanks to this method, not only the error arising from the difference of dispersion, but even if desirable a certain degree of impurity of the spectrum, may be easily done away with and the maximum intensity of light obtained. I may add that I still prefer using a pure spectrum.

The experiment undertaken to find the relative effect of the two halves of the spectrum was conducted in the following manner. A beam of light reflected by a large Foucault heliostat (furnished by M. Pellin) (fig. 9), and condensed on the slit by means of a lens of 25 cm. diameter, was decomposed by a direct-vision prism and recomposed into these two complementary bands of yellow and blue light. If we admit that the limits of the effective rays in the visible spectrum correspond to the wave-lengths of 700 and 400 millionths of a millimetre, a line passing somewhere about the wave-length 550

will divide our spectrum into two halves with reference to the
diffraction spectrum.   The analytical results obtained may conse-
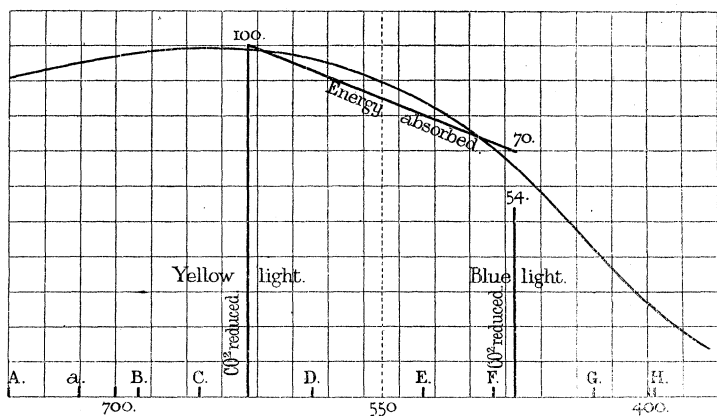quently be directly compared and plotted on a diffraction spectrum.

They may be stated thus : if the quantity of carbon dioxide reduced
in the yellow half be represented (as a mean of six experiments) by
100, the effect of the blue half will be 54 (fig. 6B).   The principal
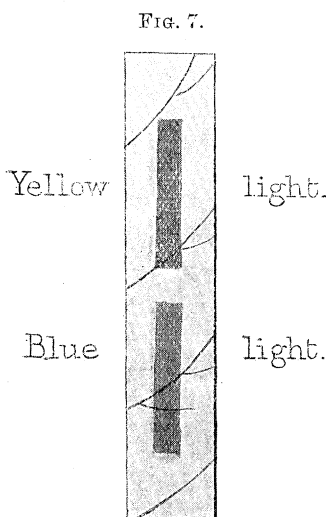
Fig. 6.

A.
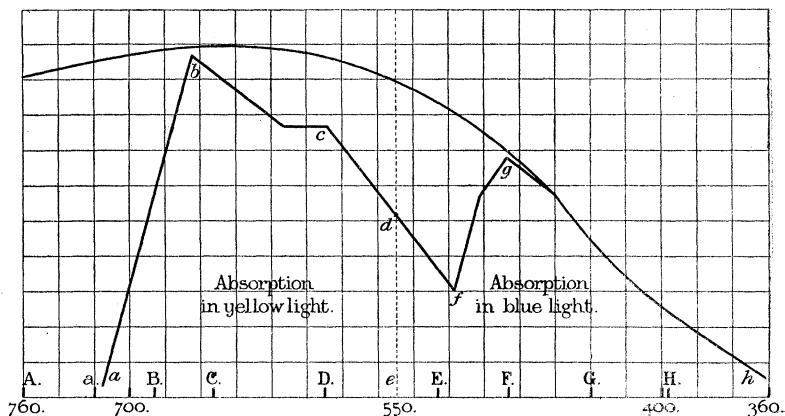
B.

result is that the effect of the blue and violet rays has been till now
somewhat underrated.

The same result has been obtained with regard to the iodine starch
test, the spectrum being divided as just described in the horizontal
sense in order to have the two surfaces directly superimposed (fig. 7).

Whilst in the spectrum as we have just seen (fig. 5), the blue and violet rays produced hardly any effect, here the effect of these rays was quite prominent.

The fact that the reduction of carbon dioxide as well as the production of starch is due to the rays absorbed by chlorophyll, may be thus considered as fully established in all its details, the more so that an elaborate bolometric study of the chlorophyll spectrum in the infra-red by Donath, has proved that there are no absorption bands in this region. This accounts for the fact established, as we have seen, by Cailletet, that no reduction can be attributed to the rays filtered through Tyndall's iodine solution.

Fig. 7.

Yellow light.

Blue light.

We have now to consider the second of the two points mentioned above concerning the connection between the photo-chemical process and the absorption of light. We have seen that Jamin, Edmond Becquerel and, lastly, Lommel, expressed the opinion that Herschel's law might be applied to our case. But it seemed to me that in this reasoning there was a certain logical flaw, a link missing, between the premisses and the conclusion deduced. Herschel's law means that the photo-chemical effect is confined to those rays of light only which are absorbed by the substance undergoing chemical change. Sir John Herschel applied it himself to chlorophyll, showing that this substance underwent a process of bleaching in exactly those rays of the spectrum which correspond to the absorption bands.

But in the reduction of carbon dioxide we have quite a different case—the substance undergoing decomposition is a colourless gas and light is absorbed by another substance, chlorophyll. It was decidedly impossible to see in this case a direct application of Herschel's law, and that was one reason the more for my not insisting on this point at the beginning of my researches. But the same reason accounts for my being one of the first to acclaim the importance of Professor Vogel's beautiful discovery of *optical* sensitisers.

This brilliant achievement not only revolutionised the practice of photography but furnished at the same time that missing logical link, the absence of which did not permit of Herschel's law being applied to the case of the green leaf. Vogel's researches on Eosine were shortly followed by Edmond Becquerel's experiments on collodion plates

sensitised by an admixture of chlorophyll, so that in less than a year after the publication of my first experiments I was able to give them a rational explanation in the light of Vogel's new theory. This idea, that chlorophyll plays in the living organism the part of an optical sensitiser, which I advanced for the first time in 1875, is now generally accepted, but I suppose very few botanists have had an opportunity of seeing the photographic effect of chlorophyll on a sensitised plate. The late Edmond Becquerel in 1877 kindly showed me his beautiful collodion plates; but the collodion process being now somewhat out of fashion, I tried to find a simple way of applying the sensitising action of chlorophyll to the gelatino-bromide plates now in general use. Alcoholic solutions are not practicable in this case, and I found it very convenient to substitute a potassium or sodium chlorophyllate, which is easily soluble in water.

Here is a plate (Ilford ordinary), half of which has been immersed in a bath of chlorophyllate of sodium, and then exposed to a spectrum reduced by means of a yellow filter to its least refrangible part (fig. 8).

FIG. 8.

B. C.   D.
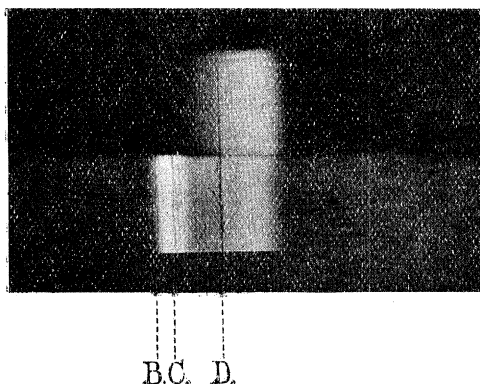
It may be seen that whilst on the ordinary plate the effect stops short of the line D., in the part that has been immersed in the sensitising bath there is a bright band corresponding exactly to the principal absorption band of chlorophyll between the lines B. and C.

I tried to push the analogy between the effect of chlorophyll in a sensitised plate and in the green leaf a step further, by showing that chlorophyll is an optical sensitiser, not only in Vogel's but in Sir William Abney's acceptance of the word.

Sir William Abney admits that an optical sensitiser is as a rule a fugitive dye, *i.e.*, one that rapidly fades in the light. Sir John Herschel's classical researches on the bleaching of paper tinged with chlorophyll, prove that chlorophyll belongs to the above class. I have

here a chlorophyll print of a fern leaf. The leaf was applied to a plate coated with a film of collodion tinged with chlorophyll. After a short exposure to direct sunlight we observe that the whole ground is bleached, the parts protected by the leaf retaining their original hue. The image is fixed by a short immersion in a bath of copper sulphate. On submitting such chlorophyll collodion plates to different rays of the spectrum, I convinced myself that this bleaching effect is due to the same rays which effect the reduction of carbon dioxide.

I may perhaps be allowed to dwell a little longer on this important question of chlorophyll playing the part of a sensitiser. The sensitisers are in general divided into two groups—chemical and optical. The former are considered simply absorbents of one or more of the products of the reaction, the latter are at the same time absorbents of radiant energy. An optical sensitiser is supposed to be at the same time a chemical sensitiser, but the reverse of course does not hold good. The existence of chemical sensitisers was admitted long before Vogel's discovery; many instances of their action may be found in Becquerel's well-known book, and in consequence at a very early date, in 1871, in my Russian work "Spectrum Analysis applied to Chlorophyll," I admitted that chlorophyll may be considered a sensitiser in the purely chemical acceptance of the word. I insisted that the reduction of carbon dioxide being essentially a process of dissociation, and the "rapidity of dissociation depending on the removal of the products of dissociation," "the plant acts as an absorbent, continually disturbing the equilibrium between the carbon dioxide and the products of its dissociation," and finally that we must probably admit "the existence of two modifications of chlorophyll somewhat corresponding to hæmo- and oxyhæmoglobin of the blood, the latter being perhaps capable of originating a product analogous to carbonyl hæmoglobin."

At the time it seemed to me that these two states of oxidation corresponded to the normal green chlorophyll and to Sir George Stokes's modified chlorophyll (fig. 2), my experiments having put it beyond doubt that the latter was the product of oxidation of the former; but later I discovered another reaction of chlorophyll, which I look upon as highly important from the physiological point of view we are now considering. When a moderately concentrated solution of chlorophyll is acted upon by hydrogen in the nascent state, it is transformed into a substance nearly colourless, or of a pale yellow hue, but possessing a beautiful purple colour when highly concentrated. This substance can exist only in a total absence of oxygen. On being brought into contact with air it almost immediately recovers its natural green colour. The spectrum of this reduction product of chlorophyll (I proposed to call it protochlorophyllin, or simply protophyllin) in a diluted state, is characterised by a total absence of bands in the less

refrangible part of the spectrum, but when sufficiently concentrated, it shows a very distinct band nearly corresponding to the II band of chlorophyll and another band about the place of the IV, no traces of the principal band I being present. It was the first case of a product of chlorophyll characterised by the total absence of what had been considered the principal characteristic of the chlorophyll spectrum. It is evident that we have here a reduced constituent of chlorophyll, from which chlorophyll is regenerated almost instantaneously on its being brought into contact with the oxygen of the air.

Having obtained these important reactions of reduction and regeneration of chlorophyll in its solutions, I took all possible pains to find out the existence of this protophyllin in the living plant. The formation of chlorophyll in etiolated seedlings being a process of oxidation (the fact was demonstrated in my laboratory by Dementieff in 1873), it was quite natural to search in etiolated seedlings for this protophyllin so easily convertible into chlorophyll on being oxidised. The facts exposed in the current literature were rather discouraging, the so called *Etiolin* of the German botanists always presenting the characteristic chlorophyll spectrum. My first steps were also unsuccessful. I obtained, it is true, solutions where the second band was rather more pronounced ; in some cases it had even the same intensity as the first band, but I looked in vain for solutions in which the first band would be totally absent, until the idea struck me that the precautions generally taken were not sufficient to eliminate completely the influence of light. I inclosed the small pots with the seeds that were intended to germinate (sunflower seeds proved to be best) in tin cases, which in their turn were kept in a cupboard in a photographer's dark room. The solutions were prepared in the same dark room, all the precautions used in orthochromatic photography being taken. The result was that I obtained solutions of protophyllin without the least traces of the chlorophyll spectrum.

The colouring power of these solutions being very small, they had to be studied in tubes 50 cm. long. In general about ten or twenty cotyledons were used for preparing the necessary solution for filling the tube. But it was sufficient to expose a single cotyledon, or even a part of one, for an instant to the light in order to obtain a solution that would present the characteristic chlorophyll bands. These facts suffice to prove that in the living plant there exists a colouring matter with the properties of reduced chlorophyll, almost instantaneously convertible into chlorophyll on being exposed to light.

Of course it would be of still greater importance to obtain a direct proof of the presence of this reduced constituent of chlorophyll in the green leaf ; this fact alone could account for its playing the part of a chemical sensitiser ; but the detection of this substance in the green leaf must needs present certain difficulties. As I have just insisted, the

solutions of protophyllin possess a colouring power greatly inferior to that of chlorophyll, so that their presence may be easily disguised by the latter. Thus assuming that this reduction of chlorophyll actually takes place in a green leaf, it will hardly be manifested by a change of colour or of the spectrum, but simply by a diminution in the intensity of the green hue, by a certain bleaching of the green parts exposed to the light. Now this bleaching effect has in fact been often observed, but was generally attributed to the migration or change of volume of the chloroplasts—a supposition that seems to me highly improbable when applied to the palisade-parenchyma, especially when the rays of light do not fall strictly in the direction of the cell axes. I really think that at any rate the greater part of this bleaching may be accounted for by supposing some of the chlorophyllin to be reduced to protophyllin. To sum up, we may thus admit the existence not only of two, as in the blood, but even of three states of oxidation of the green colouring matter. These transformations may be considered a starting point for a theory of chlorophyll as a chemical sensitiser.

Having enumerated the chief arguments in favour of the admission that chlorophyll may be considered a sensitiser in both acceptances of the word—a chemical and an optical sensitiser, an absorbent of the products of reaction as well as an absorbent of the active rays—I will try to take a step further and show that it may be considered not only one of the innumerable representatives of the group, but a substance quite exceptionally adapted to this function.

At the very outset of my researches I was impressed with the idea that such an intensely endothermic reaction as the dissociation of carbon dioxide, must needs stand in some relation to the energy of the radiations involved in the process. At the time we had no direct knowledge of the distribution of energy in the spectrum, but I pointed out that researches in the diffraction spectrum might possibly alter the current notions concerning the relative calorific effect of the different radiations and prove that the greatest energy should be attributed, not to the dark infra-red rays, but to a certain group of rays in the visible spectrum—possibly the same that are absorbed by the green plant, so that the two maxima—of energy and of chlorophyll absorption—may after all coincide.

My previsions were fully confirmed by the brilliant researches of Mr. Langley and Sir William Abney. I called attention to this fore-seen coincidence in a small note in the 'Comptes Rendus' shortly after the appearance of Mr. Langley's classical paper. My statements of these facts being still contested, the correctness of my quotations being even suspected by Professor Engelmann, I feel myself bound to bring forward the original data. In Sir William Abney's paper in the 'Proceedings of the Royal Society' for 1887, it is shown that the maximum of energy corresponds to the wave-length about two-thirds between 600 and

700 millionths of a millimetre, let us say 666 (a figure easily retained
in the memory, it being the number of the Beast).   The maximum of
absorption is somewhere about the two-thirds between the lines B.
and C., between 686 and 656, consequently again about the wave-length
666-millionths of a millimetre.   At a later date Mr. Langley furnished
data which I have plotted above (fig. 6, A and B), and we shall soon
have occasion to apply them. It must be remembered that Mr. Langley's
numbers refer to what he calls "high sun."   For the mean intensity of
the whole day the coincidence would probably be still closer.   We may
consider the same question from another point of view.   I have made
a rough attempt to represent the relative amplitudes of the vibrations
corresponding to the different rays of the spectrum, and it may be seen
that the greatest disturbances correspond to that region of the spectrum
where the principal absorption band of chlorophyll, and therefore the
maximum chemical effect on the carbon dioxide, are situated.

But we may perhaps find a still more convincing argument in favour
of the supposition that the photo-chemical effect of a radiation is a
function of its energy, in a comparison of chlorophyll with the other
sensitisers, and still more in a comparison of the relative effects of
yellow and blue light in the experiment just cited.

There is an important fact, based on the testimony of such able
observers as Eder and Vogel, and made especially conspicuous in a very
elaborate paper of Mr. Acworth, the fact that the two maxima, the
maximum of absorption of light by the sensitiser and the maximum of
photograghic effect on the sensitised plate, do not strictly coincide—
the latter, as a rule, being shifted towards the red end of the spectrum.
No explanation has been put forth for this fact, nor does Mr. Acworth
offer any, holding at the same time that the fact in itself is past any
doubt.   But so far as chlorophyll is concerned a curious error seems to
have crept into Mr. Acworth's statements.   He admits that chlorophyll
makes no exception to the general rule, *i.e.*, that the maximum of
chemical effect does not correspond to the maximum of absorption in
the first band but is shifted to the red end of the spectrum—though an
attentive glance at the figure relating to chlorophyll will easily convince
one that there is something wrong about it.   As in the other cases,
Mr. Acworth gives the two curves, the absorption curve and the curve
of the sensitising effect on the photographic plate, in order to show
the shifting of the latter towards the red end of the spectrum.   But
in fact his statement (in both text and figure) concerning the absorp-
tion spectrum of chlorophyll is inexact.   Here are his very words :
" Die Absorption dieser Emulsion ist, was die Haupt- und weniger
brechbare Bänder betrifft, deutlich ausgeprägt.   Dieses beginnt etwas
vor C., erreicht bei $C\frac{1}{3}$. D. ein Maximum ; jenseits desselben ist die
Absorption nicht hinlänglich scharf zu verfolgen, um sie genau zeichnen
zu können."

Now, it is a fact testified by thousands and thousands of observations that the principal chlorophyll band lies between B. and C. and not between C. and D. On the contrary, Acworth's curve representing the sensitising effect has its maximum between B. and C.—nearer to C.—exactly where innumerable observations unanimously place the principal absorption band of chlorophyll, and in this respect all we know only confirms the exactness of Mr. Acworth's experiment. Beginning with the late Edmond Becquerel's first experiment and ending with my recent photographs, we always see the maximum of the sensitising effect corresponding to the band between the lines B. and C. It follows that Mr. Acworth places the maximum of photographic effect in the right place, but the maximum of absorption in an evidently wrong place, and it is only by means of this evident error that chlorophyll is made to agree with the general rule.

In reality the two points perfectly coincide, and as a further consequence chlorophyll must be considered an exception to the general rule of the shifting of the maximum of chemical effect towards the red end of the spectrum. Now it seems to me that the exception as well as the rule might find an explanation in the simple admission that the photo-chemical effect must be attributed not only to the degree of absorption of a certain group of rays of which the band is composed, but at the same time to the energy or the amplitude of the corresponding vibrations. In every absorption band (in the limits of the visible spectrum) the side nearer to the red end will be composed of radiations possessing a greater amount of energy, and as a consequence of the supposition just made, the maximum effect must be shifted that way until we arrive at that part in the red where the maximum of energy and absorption coincide. At this point there will be no reason for any shifting of the photographic maximum, and so we get an explanation of the curious anomaly presented by chlorophyll as a photographic sensitiser.

Of course the *experimentum crucis* for testing my hypothesis would be, using Sir William Abney's wonderful method of infra-red photography, to see whether in the infra-red part of the spectrum Acworth's rule would be reversed. But this kind of research is quite out of the reach of a botanist.

Meanwhile it seems to me that the facts we have just seen concerning the relative effects of yellow and blue light, may afford a new argument in favour of this supposed relation between the chemical process and the energy of the active rays. In fact we may consider it a limiting case, since it allows us a direct comparison of the relative effects of two groups of absorption bands lying at the opposite ends of the visible spectrum, and consequently possessing the greatest difference possible as to the amount of energy of the corresponding radiations. We have seen that this relative effect was represented by 100 for the

less refrangible part and by only 54 for the more refrangible rays. But we ought to take into account the different degrees of absorption in these two halves of the spectrum. It would seem that the simplest way would be take into consideration the directly visible absorption bands presented by the colouring matter in the same state of concentration as in the leaf (we shall see later how such solutions may be obtained). This was, in fact, the way I first adopted. But this estimate must be considered as not free from error, since the eye is a poor photometer for slight and gradual differences of absorption. This is the reason why in this class of researches, as already mentioned, we must have recourse to the spectro-photometric method.

The choice of the instrument is far from being immaterial—for instance, Vierordt's photometer with a double slit, generally used by German physiologists, is not to be trusted. As the most practical form I consider d'Arsonval's spectro-photometer as furnished by M. Pellin. Here are, for instance, two photometrical curves for solutions of normal and modified chlorophyll of equal concentration. And here (fig. 6A) we have the spectro-photometric curve corresponding to the absorption of light by a single leaf of maple, such as was used for the experiments with yellow and blue light. Reduced to the normal energy spectrum the relative amounts of absorption are measured by the areas a, b, c, d, e, and e, d, f, g, h. The total amount of energy of the spectrum I take from Mr. Langley's paper on "Energy and Vision" in the 'Philosophical Magazine' for 1889. The relative values of the energy absorbed by the leaf, as represented by these areas a, b, c, d, e and e, d, f, g, h, will be 100 for the yellow and 70 for the blue (fig. 6, B). If plotted together with the previous analytical data (100 and 54) the results of these calculations furnish a new argument in favour of the supposition that the chemical process stands in direct relation to the energy of the radiation.

All things considered, it seems to me that there is a certain amount of evidence for admitting that, so far at least as chlorophyll is concerned, the photo-chemical effect of a radiation depends not only on the degree of its absorption, but at the same time on its energy or amplitude of vibrations; or, to put it in other words, that of two rays equally absorbed, the one possessing the greater amplitude of vibration will produce the greater effect. From this point of view chlorophyll, since of all radiations it absorbs those possessing the greatest energy, may be considered not only a sensitiser but perhaps the best of sensitisers, specially adapted to its function.

I feel that I cannot dismiss the subject without a word of remark concerning the attitude taken towards my researches by the German physiological school, as represented by Julius Sachs and Professor Pfeffer. The latest opinion on the subject expressed by Sachs was the following:—"Alle direkte Beobachtungen zeigen, dass das Maximum

der Sauerstoffabscheidung im gelben Licht stattfindet. Alles zusammengefasst, haben die Untersuchungen über das Chlorophyllspectrum bisher auch nicht die geringste physiologisch werthvolle Thatsache ergeben, d. h. wir würden von der physiologischen Function des Chlorophylls genau dasselbe wissen, wenn uns auch das Spectrum desselben vollständig unbekannt wäre."

It is also a well-known fact that for a long while Professor Pfeffer was my opponent and the staunchest adherent of the old theory. In the first edition of his Handbook he treated my researches in general as an experimental blunder not worthy of further notice. But shortly after the publication of his work a sudden change of scientific opinion was observed in favour of my point of view, thanks to M. Van Tieghem in France, and to Professor Sydney Vines in this country. At the same time Engelmann and Reinke—though using methods far less exact than those I had used—obtained the same results. It was impossible for Professor Pfeffer to hold his position any longer, and in the second edition of his book he practically adopts my views; but this time the conclusions, which were subject to doubt so long as they were associated with my name, are simply attributed to the two German investigators. More than that, a new theory is advanced in order to conciliate his former and present points of view. Professor Pfeffer admits the existence of two different assimilation curves : the one corresponding to the chlorophyll absorption spectrum—the primary as he calls it, and another, the secondary, having its maximum in the yellow rays. But nothing can be more easy than to prove the non-existence of this latter. In fact, if Professor Pfeffer is right, I ought to have obtained his secondary curve. But, as we have seen, I was the first to obtain the primary—the only real one—and that is probably the chief reason why Professor Pfeffer was obliged to pass over my researches in silence. His secondary curve is brought forward in order to cover a retreat imposed on him by the evidence of facts.

Having thus stated the position taken towards me, with rare exceptions, by the German botanists, I feel that I am the more bound to express my gratitude to the late Edmond Becquerel and M. Berthelot, to whom I am indebted for the appearance of my researches in the pages of the 'Comptes Rendus' and the 'Annales de Chimie et de Physique.'

Up to this point we have been considering the qualitative aspect of the problem, we have studied the photolysis or the reduction of the carbon dioxide and the photosynthesis or the production of starch, in their relation to the chemical and optical properties of chlorophyll and to the energy of the active rays. We will now consider the same phenomenon in its quantitative relation to the total amount of radiant energy incident on the green leaf, and we will try to find the law which regulates its dependence on the intensity of the radiation.

Edmond Becquerel, in his already cited work 'La Lumière, ses Causes et ses Effets,' was the first to attack the question of the storage of the solar energy in the green plant; he made the first attempt to obtain an approximate value for what Mr. Horace Brown has recently so appropriately called *the economic coefficient* of the photo-chemical process.   Edmond Becquerel arrived at a very moderate estimate. The potential energy represented by the organic matter of a culture of sunflower is only 4/1000 of the available solar energy; in a forest it does not surpass 1/1000.   A couple of years later, in 1871, I applied the same calculation to more definite physiological data and obtained about 1 per cent.   In 1876 my friend Professor N. Müller made for the first time a direct experiment, the leaf which decomposes the carbon dioxide and the pyrheliometer being placed side by side. His estimate is perhaps somewhat too high, being 5 per cent., which I think must be attributed to the fact that the pyrheliometric data seemed to be too low, when compared with the numbers generally accepted.   In 1894 I repeated the same experiment by placing the leaf and the pyrheliometer in exactly the same conditions, as will be presently described, and obtained the value $3\frac{1}{2}$ per cent.   It must be noted that in Müller's and my experiments the leaves were placed in an artificial medium, in a gaseous mixture containing from 5—10 per cent. of carbonic acid gas, this mixture giving the highest effect.   Mr. Horace Brown has more recently made a highly interesting and much more difficult determination of the same value, placing himself in more natural conditions by using a current of atmospheric air with its normal content of carbon dioxide.   The result was 0·5 per cent., but in one case (in which the air had been enriched with carbon dioxide to the extent of about 5·5 times the normal amount) the efficiency of the leaf was raised from 0·5—2·0 per cent.

Now that we are quite sure that only those rays that are absorbed by chlorophyll effect the reduction of the carbon dioxide, it is evident that this economic coefficient must principally depend on the degree of absorption of light by the green matter of the leaf.   It is useless to insist how important it is to know this fraction of the total radiation available to the plant.   The following thermoscopic method which I adopted in 1884 seems to me still the surest and simplest way of estimating this quantity.   The problem, as it presents itself in a real leaf, is highly complicated, because of the scattering effect of the cells and chloroplasts, very similar to that presented by the silver deposit in the photographic plate so ingeniously explained by Sir William Abney. But the problem may be considerably simplified, if for a real leaf we substitute what may be called a liquid leaf.

If using a steel punch we cut out of a leaf a certain area and dissolve its green matter in a volume of alcohol just sufficient to fill a glass cell of exactly the same vertical section, we obtain a liquid

layer of the colouring matter of practically the same concentration. If the absorption due to the glass and to the solvent be determined at the same time, the difference of the two quantities will give us the amount of absorption attributable to the colouring matter of the leaf. All the measurements were made by means of a particular form of thermopile devised by me in 1870 and somewhat like the one recently adopted by Rubens. The bars of the two metals are soldered in a zig-zag, so that the two rows of junctions are turned to the same side. When one row is exposed to the sunlight, the other being sheltered by a screen, we measure the effect of the direct radiation. On interposing the chlorophyll solution we ascertain the fraction of sunlight that is filtered by the solution, and lastly, by interposing the same glass cell with the solvent we find the quantity transmitted by both these media. The difference of these two last quantities corresponds to the absorption by chlorophyll. The ratio of this quantity to the one obtained in the first operation gives the fraction of direct sunlight absorbed by the chlorophyll of a leaf. These three operations may be reduced to two—the measurement of the direct radiation and that of the difference of the two liquids in a double-glass cell. Here is the apparatus arranged for me by M. Pellin in 1889 (Plate 21, A, B).

At the bottom of the cylindrical mantle lies the thermopile, protected by a whole range of double slits from sudden variations of temperature, such as might be caused by wind (Plate 21, A). The double-glass cell, enclosed in a metallic box furnished with two shutters, slides in a groove in front of the outer slits (Plate 21, B). When we performt he first operation, one of the slits is shut by the metallic box (both shutters, of course, being also shut); when performing the second operation the box is made to slide so as to shut both the slits, the shutters being open.

I proposed to call this apparatus a *Phytoactinometer*, since it gives us the direct measure of the fraction of solar energy available to the plant in the most important of its functions.* Here are some of the values for a single leaf and for three leaves. Three is the maximum number of leaves that may be superimposed with any profit to the plant, the light reaching the fourth leaf being, as has been proved by Professor Müller, already deprived of its chemical activity.

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* This simple thermoscopic method is in most cases preferable to the far more troublesome method of spectrophotometry.

Absorption of Solar Energy by the Chlorophyll of Leaves.

Direct Sunlight.

Maple, 1 leaf   (mean of 32 expts.)............... 27 per cent.
   ,,   3 leaves (,,   4 ,,   )............... 31   ,,
Lime, 1 leaf   (mean of 6 expts.)..... 29   ,,
   ,,   3 leaves (,,   3 ,,   )................. 35   ,,
Oak, 1 leaf (mean of 4 expts.) .... 23·5   ,,
Plantain, 1 leaf .... 23·4   ,,
Potamogeton lucens, 1 leaf (mean of 8 expts.) ... 20   ,,

Yellow Light.

Maple, 1 leaf (mean of 8 expts.) .... 13·8   ,,

Red Light.

Maple, 1 leaf 10   ,,

Before applying these values to a full discussion of the question of the economic coefficient and maximum chemical efficiency, the second of the problems we have just stated—the influence of the intensity of light—must be studied. It is too often admitted that the photo-chemical effect stands in direct proportion to the light intensity, though the contrary has been put beyond any doubt in some cases where most exact methods have been applied. I have but to mention the masterly researches of Sir William Abney on photographic plates.

The influence of the light-intensity on the reduction of carbonic dioxide has been studied by Wolkoff, Van Tieghem, Kreussler, Famin-tzin, and Reinke, but the results being very discordant and difficult to reconcile, I undertook a somewhat detailed study of the question. The methods adopted were the following (fig. 9). In a dark room a pencil of light was obtained by means of a powerful Foucault heliostat (the mirror measuring 18 cm. by 40 cm.) and a lens 25 cm. in diameter. Parallel to the axis of this cone of light was placed an optical bench 2 mm. in length with a scale divided in centimetres, and furnished with five movable and adjustable oblong stands placed across the bench. On each of these stands was placed one of those apparatus with pieces of leaves already described. The distances on the bench were so chosen that the relative intensities of light corresponded to the numbers 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, the unit being direct sunlight 3 or 4 hours on each side of noon, when the variation of intensity (as may be seen in the adjoining diagram, borrowed from Crova) is not very consider-able. Any experiment, thanks to the gasometric method already described, did not last more than 20 minutes, so that one could be sure of disposing of continual sunshine.

This disposition being rather troublesome, as it implies the use of a dark room and of such expensive and cumbersome apparatus as the large heliostat and the optical bench, I devised later the following more simple and portable form (Plate 22). This time it was the law of the cosines that was applied to measure the intensity of the incident rays. A small metallic platform movable on a vertical axis,

Fig. 9.
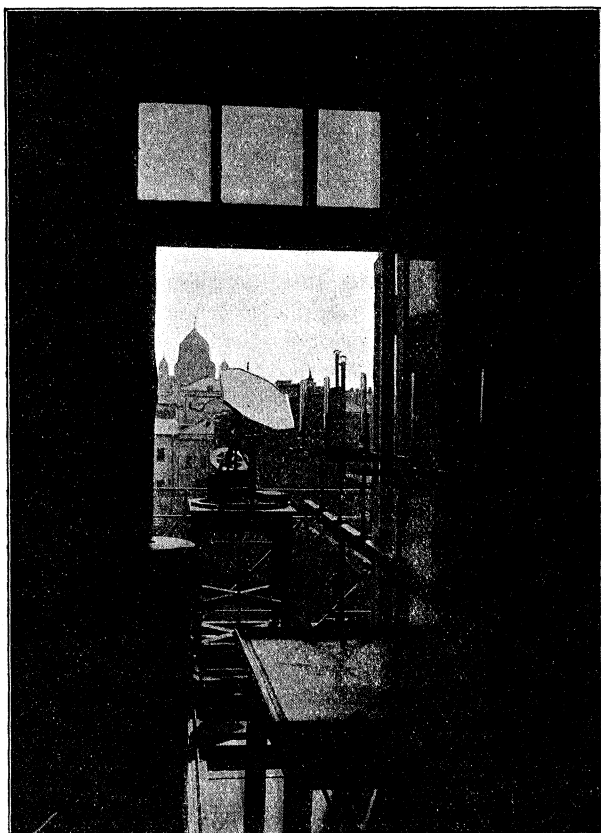
and at the same time easily adjustable in the direction of the sun-beam, bears four smaller movable platforms with four glass tubes containing leaves. The four platforms may be put at such angles that the light received by the leaves will vary in the same ratio $1, \frac{1}{2}, \frac{1}{4}, \frac{1}{8}$ (Plate 22, A). As the experiment lasts only 15—20 minutes, the observer standing by may re-adjust three or four times the position of the large platform, so as to keep the glass tube of the first small plat-form at right angles with the falling sunbeam. The loss of light due

to reflection and absorption by the glass of the tubes at different angles must be taken into account, and for that reason I prefer using in this case these tubes specially prepared for me at the well-known glass manufactory of Leibold in Köln (Plate 22, B). They are made of plates of mirror-glass luted together. A sample of the same plate is tested photometrically, being placed before a photometer on a divided circle.

The third method was an application of my micro-eudiometer to the measurement and analysis of the bubbles of gas given off by an aquatic plant, a Potamogeton or Elodea. The quantity of gas produced in 1 minute is sufficient for one analysis, so that the same plant in the course of 10 or 12 minutes may be placed in the different points of the cone of light. Of course this method does not aspire to the same degree of precision as the two former, but its defects are but a consequence of the aquatic habits of life of the plant. On the other hand, it has the great advantage of being applied to the same object, all individual differences being eliminated, and the interval of time being so short that one may be sure of the intensity of light remaining invariable during the four consecutive operations.

On the whole, the results obtained by these different methods on different plants at different times were concordant and may be summed up in the form of the following curve—100 being direct sunshine at about noon, the rays falling perpendicularly on the surface of the leaf (fig. 10).

1. If the whole range of intensities is taken into account direct proportionality is quite out of the question. We may add that Reinke pushed his experiments still further in the direction of higher intensities. Though he used the old method of counting the bubbles, still for such rough approximations his results may be relied upon. On exposing his plant in a convergent cone of light to intensities amounting to two, four, eight times the intensity of direct sunlight, no corresponding rise of the chemical effect could be observed, the curve remaining parallel to the abscissa.

2. The maximum effect is obtained, roughly speaking, at about *one-half* of direct sunlight.

3. Up to a certain degree of intensity the effect may be considered proportional to this intensity. Past a certain point of the curve, for a further rise of intensity there is no corresponding increase of chemical effect.

We must now try to find the reason of this general form of the curve. But before entering on a discussion on this subject, we must first take into consideration one point more, concerning another kind of work produced by the radiant energy absorbed by the chlorophyll of the leaf. Professor Wiesner has put it beyond any doubt that a considerable part of the evaporation going on in the green leaf is accomplished at the expense of the radiant energy absorbed by

chlorophyll.   It has since been proposed to denote this part of the
process by the special name of chlorovaporisation.   My old friend the
late Professor Dehérain pointed out a highly interesting fact, subse-
quently confirmed by Professor Sorauer and especially by M. Jumelle,
that these two principal functions of the leaf—the assimilation and
the chlorovaporisation—are in a certain sense complementary, the
evaporation falling off in the presence of carbon dioxide.   This result
would seem quite natural, since the same source of energy must be

Fig. 10.
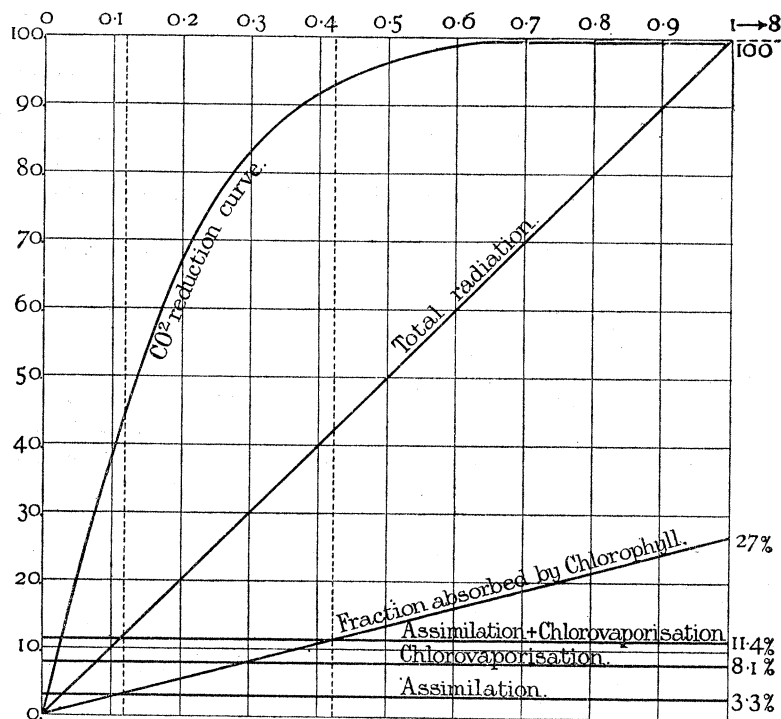
divided between the two processes—had we not known how great is
the excess of radiant energy incident on the leaf.   But now that we
know the available fraction of this total amount, we may try to
compare in a rough estimate the quantities of energy absorbed by the
leaf with those spent in the chemical work of assimilation as well as in
the physical work of chlorovaporisation.

   We admitted that the absorption of the chlorophyll of a leaf amounts
to 27 per cent.   With the diminution of the total energy of the radia-
tion it will fall off in proportion (fig. 10).   We have seen further that

the maximum economic coefficient of assimilation in our case was 3·3 per cent. But besides this chemical work there is the physical work of chlorovaporisation going on. In 1894 I made a series of evaluations of the quantities of water evaporated under the same conditions in the same tubes over mercury. Given the conditions of the experiment—evaporation in closed vessels in a saturated atmosphere, one may be sure that the greater part of the process must be attributed to chlorovaporisation.*

This line here (fig. 10) represents the expenditure of total energy due to chlorovaporisation. It amounts to 8 per cent. This third line represents the sum of work both chemical and physical done in the leaf. It will cross at a certain point the line of available energy and, beginning from this point, the whole available energy would be spent—transformed into work—and consequently the two processes, the chemical and the physical, must interfere as in Dehérain's experiment. Past this point, and of course somewhat earlier, since in no case may the economic coefficient be expected to rise to 100 per cent., the leaf will consequently be short of energy for maintaining both processes at their maximum, and the curve of the chemical process must therefore sink rapidly with the decreasing intensity of the light. At this other point of intersection the whole available energy will not suffice to keep at its maximum the process of assimilation alone. These considerations, it seems to me, give us a sufficient explanation for the first part of the curve.

I may add that these results are in full accordance with the beautiful experiments of Mr. Horace Brown and Mr. Escombe. In direct sunshine they found the economic coefficient was but 27 per cent. When the intensity was reduced to about one-tenth, the economic coefficient rose to 95 per cent., nearly the whole being transformed into work.

The next point to be considered, the reason for this form of the upper part of the curve, is somewhat more perplexing. The simplest supposition explaining this course of the process would be to admit that past a certain point the radiant energy incident on the leaf will be in excess. But can it be really admitted that the solar energy may be in excess, remembering always the very modest figure representing the economic coefficient of chemical work?

The chemical process going on in the green leaf is very often considered as somewhat paradoxical. Indeed it takes place at ordinary temperatures, whilst the principal chemical phenomenon which underlies it—the dissociation of carbon dioxide—can be realised in our laboratories only at very high temperatures, amounting to thousands and thousands of degrees. Here is a diagram of Le Chatelier's representing the dissociation of carbon dioxide in its relation to temperature and pressure (fig. 11). But this paradox is of course only apparent, and if

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* I have since made some experiments with variegated leaves of Aspidistra.

Fig. 11.

Variation de la dissociation avec t et P.

Températures.
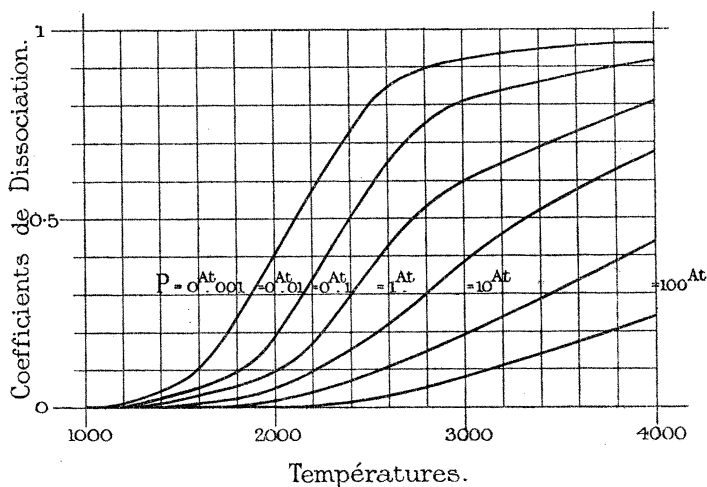
I am not mistaken, it was Count Rumford who showed for the first time that what he called the chemical properties of light may be attributed to its heating power. In his paper " An Enquiry concerning the Chemical Properties that have been attributed to Light " we find the following passage : " The heat generated by the absorption of the rays of light must necessarily at the *moment of its generation* at least, exist in almost infinitely *small spaces*; and consequently it is in bodies that are *inconceivably small* that it can produce *durable effects* in any degree indicative of its extreme intensity." At a still earlier date, in his paper " On the Propagation of Heat in Fluids " Count Rumford even applies this principle of *small spaces*, of the small sphere to which photo-chemical action is restricted, to the case we are now considering. Here are his words : " Will not the admission of our hypothesis respecting the intensity of the heat which is supposed to be generated where light is stopped . . . assist us in accounting for the production of pure air in the beautiful experiments of Dr. Ingenhousz, in which the green leaves of living vegetables are exposed immersed in water to the sun's rays ?"

By *small spaces* Count Rumford probably meant molecular spaces, but it seemed to me that it would be highly interesting to test his hypothesis in a rougher way. Now that we are perfectly sure that the phenomenon takes place in the chloroplast, and is due to the rays absorbed, we may try to make a rough estimate of the sphere of action of the sun's rays. The necessary elements of these calculations are the following : the total amount of the radiation as measured by the pyrheliometer ; the total area of Langley's energy curve ; the fraction

of this area corresponding to the absorption spectrum of a single chloroplast, and lastly the thickness of the layer of the colouring matter in this chloroplast.

The total amount of energy is established by Crova's, Langley's, Savelieff's and other researches. I have myself made some evaluations with Crova's portable actinometer. I think we shall be nearest to the truth by adopting the number 1, 3 calories per centimetre per minute.

The fraction of the total energy corresponding to the absorption of the chloroplast was obtained in the following manner. A Sorby-Browning microspectroscope was used without its prism, simply as a colorimeter, in order to obtain a solution possessing the same degree of concentration of the colouring matter as in the chloroplast).* This solution was then studied spectrophotometrically, using d'Arsonval's spectrophotometer in the way already described.

Concerning the last point—the thickness of the layer of colouring matter—I fear I shall be somewhat at variance with the current ideas adopted by histologists. It is generally admitted that the green colouring matter is distributed through the whole body of the proto-plast, but many years ago Nägeli, using the following very simple expedient, proved that it is restricted to its surface. If water be added to a preparation (Nägeli used chlorophyll grains of Clivia and Aspidistra), the chloroplasts burst, and we may see that the green shell or film has for the greater part the appearance of two valves. I have many times repeated Nägeli's experiment with the chloroplasts of Phajus, which are far more convenient, being much larger. The chloroplasts liberated from their cells are examined in a solution of sugar, where their appearance is not in the least altered. On the solution being diluted with water, at a certain moment they burst. Adding some sulphate of copper we arrest the process, and obtain durable preparations that may be dyed with Eosine and then present a very elegant object, somewhat recalling the bud of a pink poppy with its bivalved calix.

Another way of testing the distribution of the colouring matter is to inspect the chloroplasts in red light; we may then see the so-called *grana* as very small black specks in a very thin layer restricted to the surface. If we try to estimate the thickness of this green layer or film —a strict measurement is out of the question—we arrive at the approximate value of $\frac{1}{10}$ of a micron.

Having obtained all these necessary data, we may arrive at our rough estimate of the temperature that could be realised in a minute in this layer of chlorophyll, admitting, of course, that the whole energy would be accumulated without being re-radiated. We arrive at the astounding figure of about 6,000 degrees.

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* In actual fact this concentration was reduced to one-half—the reason for this reduction will be seen hereafter, when the structure of the chloroplast is discussed.

I know by my own experience that it is rather difficult to admit such formidable figures. But some analogies may perhaps help us in this respect. If we strike a flint with a bit of steel in a direction normal to its surface we obtain but a very moderate elevation of temperature, but if we hit it in the right tangential direction, so as to concentrate the whole expended energy on a small particle, it is easily brought to incandescence. Or adopting an analogy much nearer to our case; with a condensing lens we obtain temperatures amounting to thousands of degrees* by increasing the effect in a plane vertical to the falling rays. But we may arrive at the same result by a condensation of the effect in the direction of the falling ray, by diminishing the thickness of the absorbing layer. It has indeed been calculated that a platinum foil not thicker than 1/500 of a millimetre, might be made to melt on being exposed to sunlight, the effect of re-radiation, of course, being eliminated. Practically the reduction of the thickness of the absorbent metallic parts is the plan adopted for augmenting the sensibility in all modern thermoscopic apparatus—the bolometer, the thermopile of Rubens and others. I suspect that it was also the plan adopted by the plant, so that we have one occasion more to repeat with Mr. Horace Brown that the plant seems to know more about physics than we are ready to admit.

Of course these high temperatures, as such, are out of the question, and if I dwell on the corresponding thermometric equivalent of radiant energy accumulated in so small a space and in so short a time, it is only in order to show the analogy with the dissociation process so well studied by chemists. In reality we probably have here the converse of what Wiedemann calls *chemi-luminescence*, that is a direct transformation of radiant energy into chemical work without the transient intermediate stage of a high temperature. In this way the cumulative or integrating effect so characteristic of the photo-chemical process is easily understood. When the carbon dioxide is absent, the radiant energy which can no longer be directly transformed into chemical work is used as heat in the process of chlorovaporisation.

Returning to our curve, which represents the connection between the intensity of light and the quantities of carbon dioxide dissociated in the leaf (fig. 10), and comparing it now with Le Chatelier's dissociation curves (fig. 11), we may perhaps admit that their likeness, which cannot fail to strike one, might be attributed to the same cause—that about a certain point where the curve takes a horizontal direction, the temperature in the chlorophyll film—if I may be allowed, for the sake of analogy, once more to use this term—that the intensity of the radiation accumulated in this small space, is so high that the dissociation of carbon dioxide will be complete. As a consequence, past this

* My colleague, Professor Zserassky, has obtained in this way temperatures amounting to thousands of degrees.

point any further increase of intensity will be of no avail and the curve must remain parallel to the abscissa.

Of course, I do not pretend that the two values are strictly concordant, but still I think that they are of the same order of magnitude. At all events in this thin film of chlorophyll where an effect corresponding to a very high temperature may be realised, the surrounding space remaining cold, the molecules of the carbon dioxide must be in a state most favourable for their dissociation, resembling very much that furnished by St. Claire Deville's classical *tube chaud et froid.*

These considerations on the extreme tenuity of the chlorophyll film lead to other conclusions the importance of which cannot be exaggerated. For the first time we get an approximate idea of the state of concentration of the colouring matter in the chloroplast. If we take for a unit the concentration of a solution presenting in a sheet of 1 cm. the typical emerald-green colour and the characteristic absorption spectrum, we arrive at the somewhat unexpected result that the concentration in the natural state in the chloroplast is about four thousand times greater. Practically it is very near the solid state, and the superficial colour is nearly black. Two very important conclusions may be inferred from this fact. The first is an explanation of the total absence or very small degree of fluorescence observed in the living green organs. In a very elaborate paper Walter has amply proved that the fluorescence decreases rapidly when the concentration increases. If we take into consideration the state of concentration of chlorophyll in the chloroplast, we may easily account for this absence of fluorescence in the green organs, and at the same time we must admit that the chemical process going on in the chlorophyll molecule must be more energetic when the loss of energy due to fluorescence is at its minimum. For it is a fact, that those chlorophyll solutions which present the least fluorescence are most easily bleached on being exposed to light.

The second conclusion is perhaps of still greater importance. It has been very often repeated that chlorophyll extracted from a living plant loses its power of reducing carbon dioxide, and direct experiments are brought forward in support, in which chlorophyll solutions were put into contact with carbon dioxide and exposed to sunlight without any effect. Now we may see that the results of all these experiments cannot be looked upon as a proof, the conditions being utterly different from those in the chloroplast. The case is just as if we were to measure the absorption of light by lamp-black, and instead of using a coating of pure lamp-black we used a mixture of one part of black and 4000 parts of oxide of zinc. Chlorophyll, as we have seen, is practically black, and if we call it green, it is somewhat in the same sense in which we might call lamp-black brown, for in extremely thin sheets it is of that colour. In the solutions employed till now the

same amount of energy, which in the chloroplast is concentrated in a thin film, was distributed in a mass of liquid thousands of times that thickness. I do not mean that an experiment with chlorophyll in a state of high concentration ought to give a positive result—but it might; the experiment is worth making, and at all events the reverse is not proven. At least I do not see any reason why chlorophyll should act as a sensitiser on $AgCl$ and $AgBr$, but lose its sensitising power over CO_2. The difference is only quantitative, not qualitative.

Before dismissing the study of this intensity curve, I should like to mention some applications which it seems to me might be made of this law to certain facts concerning the geographical distribution and some biological peculiarities of plants. It is a well-known fact that in high latitudes plants require less time to arrive at the same stage of development than in lower latitudes. This is generally attributed to the greater length of the summer days. But then the sun does not stand so high in those latitudes, and so long as the chemical effect was considered proportional to the intensity of light, the explanation was evidently unsatisfactory. Now that we know that only about one-half of direct sunlight at noon is utilised, the remaining half being of no use, it is easier to understand that a longer period with a low sun is of greater importance than a shorter period with high sun.

We may take a step further and arrive at the conclusion that the great intensity of the sun's rays at noon may not only be of no use to the plant from the point of view of assimilation, but may become directly injurious, as being the cause of an excessive evaporation, in periods of drought even menacing its existence. Botanists are familiar with many curious peculiarities of structure or habits of leaves, beginning with the pubescence of the upper surface and ending with the vertical position, the rib turned towards the zenith, permanently as in the case of the compass-plant, or periodically as in many cases of diurnal sleep. All these peculiarities are justly considered adaptations reducing to a minimum the amount of evaporation. But one might well suppose that this economy of evaporation could not be realised otherwise than at a proportional loss of assimilation. In order to escape suffering from thirst the plant might be exposed to hunger. Now we may be sure that this is not the case, since about half the direct sunshine at noon is sufficient to maintain nutrition at its maximum. I have plotted (in calories) the diurnal energy curve of Crova, the curve representing the quantity of energy incident on a leaf placed horizontally, and lastly the double curve representing the quantity of energy accessible to a leaf of the compass-plant with its leaves placed in the plane of the meridian, the rib looking upwards. If we remember again that only about half the energy at noon is

available in the process of assimilation, we shall readily see that the difference of these areas for the horizontal and for the vertical leaf will not be considerable, whilst in the latter case the injurious effect of a great excess of radiant energy at high sun, when the saturation of the air is at its minimum, and the evaporation consequently at its maximum, will be spared to the plant. For lower latitudes the result will be still more favourable.

The same considerations hold good when applied to leaves having a woolly appearance on their upper surface, which may be compared to a plate of ground glass. Provided the scattering of light does not diminish its intensity to less than one-half at noon, the plant will be only the gainer in respect of a more economicale vaporation, without any injury to its nutrition.

Here I must put a stop to this analysis of the consequences that may be drawn from the study of the optical properties of chlorophyll. It seems to me that all that we know about the function of chlorophyll may be deduced from its optical properties, and this result is quite natural, since the process of assimilation of carbon is at the same time the process of *assimilation of sunlight.** The chlorophyll function may be thus considered as the cosmical function of the plant. Professor Boltzman in a remarkable address, read before the Academy of Vienna in 1886, expressed this idea in the following eloquent passage: " Der allgemeine Daseinskampf der Lebenswesen ist daher nicht ein Kampf um die Grundstoffe—die Grundstoffe aller Organismen sind in Luft, Wasser und Erdboden im Ueberflusse vorhanden—auch nicht um Energie, welche in Form von Wärme, leider unverwandelbar, in jedem Körper reichlich enthalten ist, sondern ein Kampf um die Entropie, welche durch den Uebergang der Energie von der heissen Sonne zur kalten Erde disponibel wird. Diesen Uebergang möglichst auszunutzen, breiten die Pflanzen die unermessliche Fläche ihrer Blätter aus, und zwingen die Sonnenenergie, in noch unerforschter Weise, ehe sie auf das Temperaturniveau der Erdoberfläche herabsinken, chemishe Synthesen auszuführen, von denen man in unseren Laboratorien noch keine Ahnung hat. Die Producte dieser chemischen Küche bilden das Kampfobject für die Thierwelt." From this point of view this field of inquiry on the cosmical function of the green plant is a borderland of two of the greatest generalisations of last century, associated with the names of Lord Kelvin and Charles Darwin—the principle of dissipation of energy and the principle of the struggle for life. And it is with a feeling of veneration that I recollect these words heard many years ago, at Down, from the late Mr. Darwin himself: " Chlorophyll is perhaps the most interesting of organic substances."

* " The Assimilation of Light by the Plant " was the original title of my Russian paper on the subject; quite lately I was glad to see this expression employed by Professor Ostwald in his ' Naturphilosophie.'

If we may consider our present notions on the cosmical nature of the process going on in the green plant an outcome of the brilliant achievements of modern physical science, it is only just to try and search for the first pioneers of this important notion of an intimate relation between the vegetable world and the sun, as manifested in the chlorophyll function. Full justice must be done to Senebier for having fully conceived the idea of this wonderful connection. But before arriving at this conclusion we must first give credit to a theory that is too often considered as only a curious aberration of the scientific mind. I mean the theory of Phlogiston. Of course, it was to be expected that a theory that had a Priestley or a Cavendish for its adepts, could not be so easily disposed of, and indeed we know that Helmholtz and especially Professor Odling have made a generous attempt at its rehabilitation. In order to understand the real meaning of this famous theory we have but to substitute for that ill-fated word the more familiar expression of potential energy, and we shall see how near the fundamental conceptions of this phenomenon from the point of view of this theory were to those of our own time, as, for instance, in these words of Senebier : " J'aime voir les corpuscules de la lumière se combiner dans les corps, etc. J'aime à croire qu'ils frapperont de nouveau nos yeux dans la flamme des matières combustibles ; il me semble lui voir former les résines avec lesquelles elle a tant d'affinité, les matières huileuses pleines de sa chaleur et de sa clarté, la partie spiritueuse des graines et des fruits, saturée de ses feux . . . Enfin le phlogistique, que la lumière formeroit dans les végétaux, ne serait-il pas la source de celui qui circuleroit dans les autres règnes ? "

When a botanist on a tour in the botanical garden of Geneva stops to admire the row of marble busts of famous botanists born in Geneva, Senebier in the foreground, he may feel sure that he stands at the very cradle of the physiological research of the 19th century, just as in our days if he would see the place from which will surely spring the physiological movement of the nascent century, he must bend his steps to another botanical garden much nearer to us and salute in the Jodrel Laboratory the starting point of quite a new departure on the way first trodden by Senebier and Saussure.

But in our historical retrospect we must go back another century. Senebier with his characteristic candour mentions the author to whom he was himself indebted for this fundamental notion of an intimate connection between light and matter. We must look for it in a book " written " as the author informs us, " at the desire of some gentlemen of the Royal Society and read at their meetings." We must open the third book of Newton's ' Opticks ' and read the 30th question, which runs as follows : " Quest. 30. Are not gross bodies and light convertible into one another, and may not bodies receive much of their activity from the particles of light which enter their composition ? For all
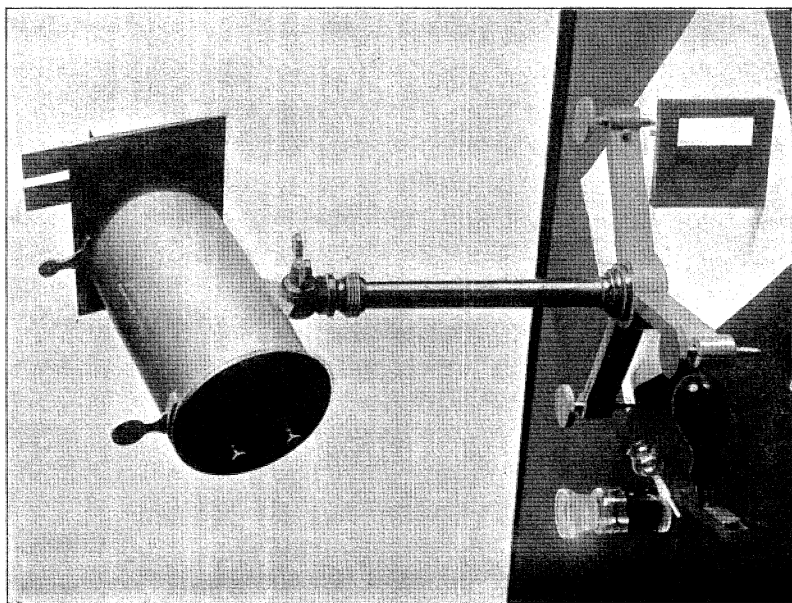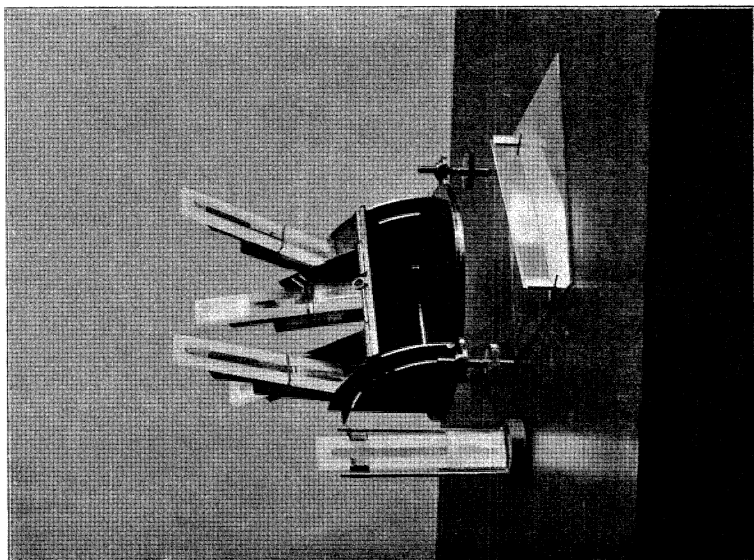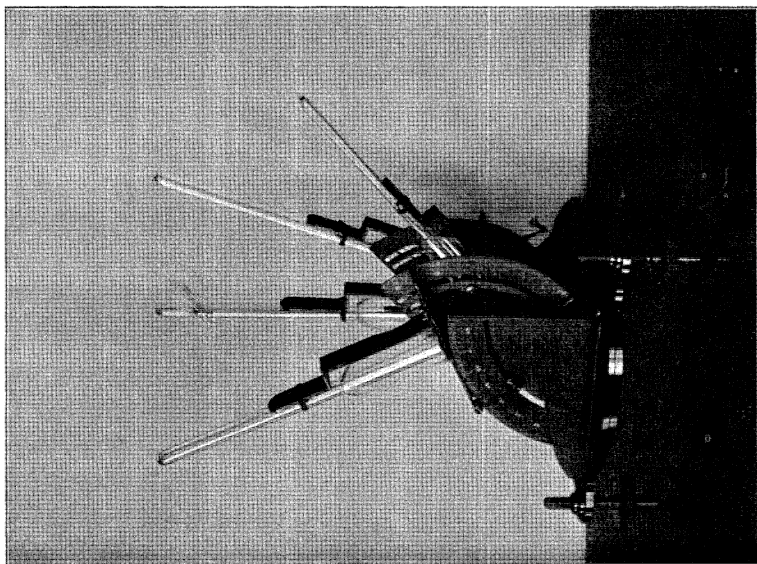
A
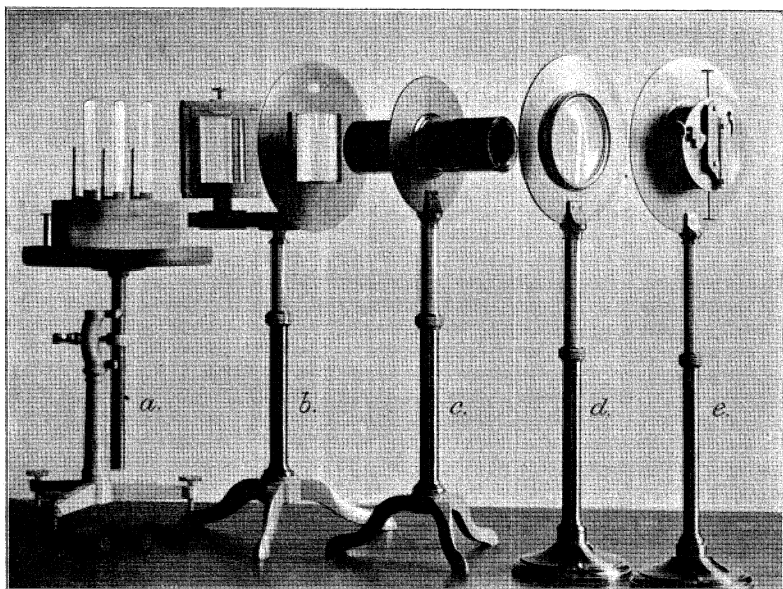
B
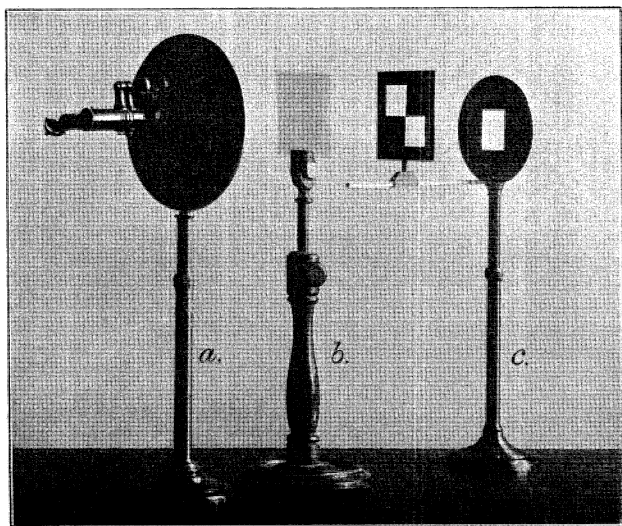
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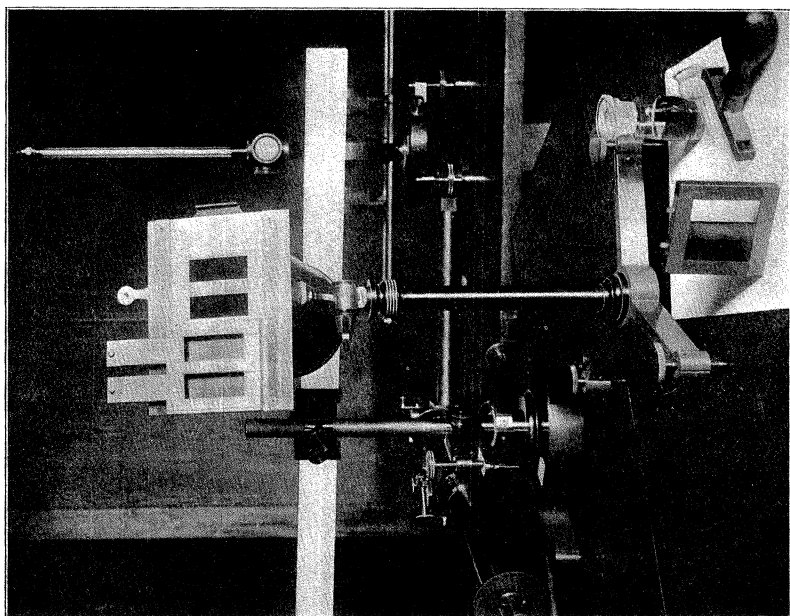
A

B

A

fixed bodies, being heated, emit light so long as they continue suffi-ciently hot; and light mutually stops in bodies as often as its rays strike upon their parts, as we shewed above." And some lines further : " The changing of bodies into light, and light into bodies, is very conformable to the course of Nature, which seems delighted with transmutations." And further on : " among such various and strange transmutations, why may not Nature change bodies into light and light into bodies ? "

If after having read these passages we turn to a micro-spectrum and see the chlorophyll-corpuscles, retaining their natural colour and trans-parency in the green and yellow rays, turn black as jet on being removed to certain regions in the red and blue, we may be sure, after all that has been said, that here we actually assist at that mysterious " transmutation of light into bodies " which makes of this black speck of matter the real link uniting that glorious outburst of energy in our central star with all the manifold manifestations of life in our planet.

It was the prism which more than two centuries ago revealed the inner nature of the sunbeam. It is the prism again which continues in our President's, in Sir Norman Lockyer's, and other able hands to unravel the mysteries of the origin of this sunbeam, and I am confident that it will still be the prism that will some day fully disclose the ultimate fate of this sunbeam on this our earth : " its transmutation into bodies."

Thus we arrive at the final conclusion that in this book of ' Opticks' we not only find the first and broadest statement of our problem in its actual state, but likewise the surest means towards its probable solution in the future. Little did Jonathan Swift suspect, when writing his envenomed satire on the Royal Society, that that which he took to be the vagaries of a madman was prophetic of Newton's immortal genius.

DESCRIPTION OF PLATES.

PLATE 20.

Apparatus for experimental research in coloured light of normal intensity (see page 436).

    A. The spectrum is recomposed into two or three vertical bands lying side by side.

    B. The spectrum is recomposed into two bands, superposed one over the other.

PLATE 21.

Phytoactinometer—apparatus for estimating the quantity of radiant energy absorbed by the chlorophyll of leaves (see p. 448).

PLATE 22.

Cosine photometer, for experimental research (see p. 450).

FIG. 1.
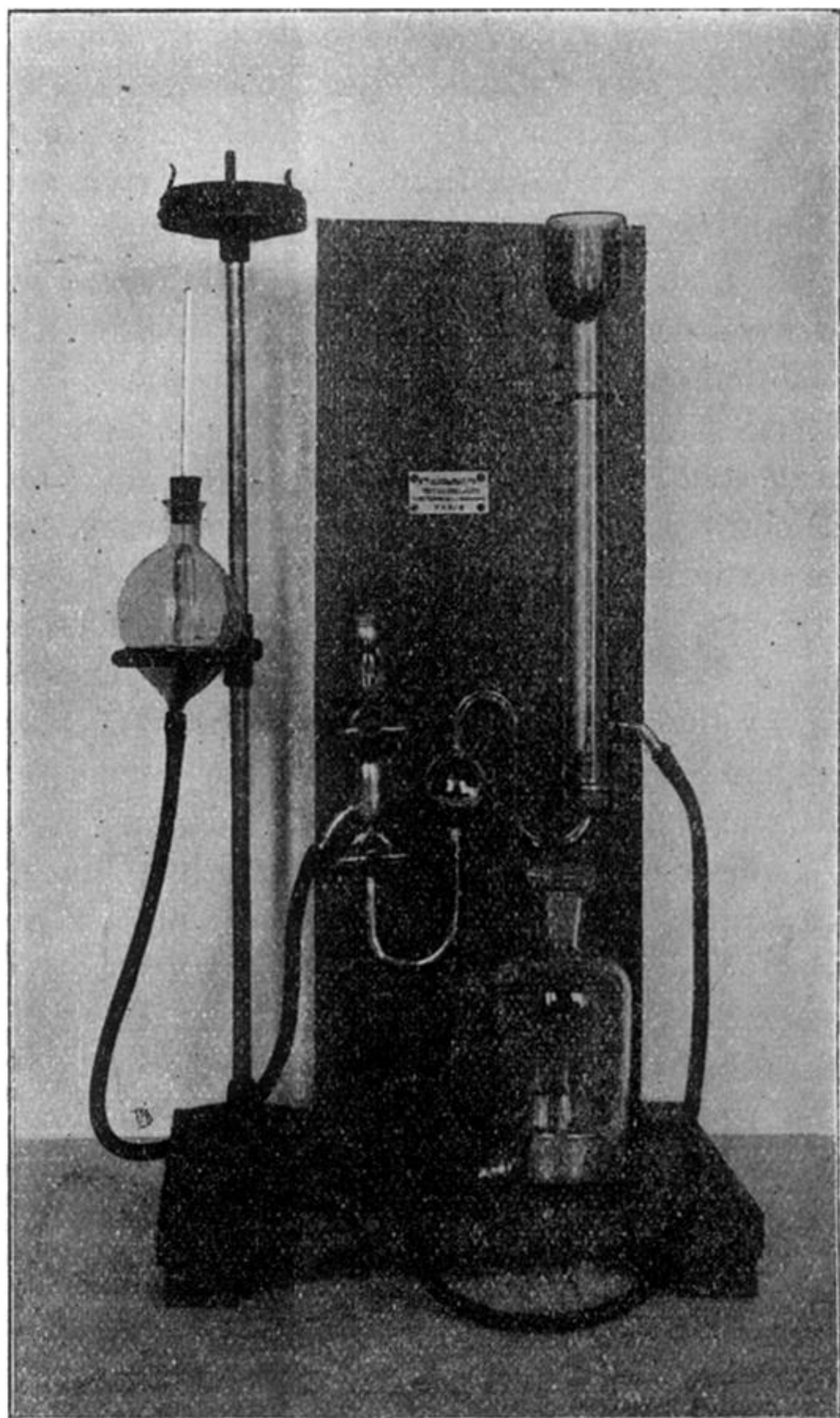
FIG. 2.
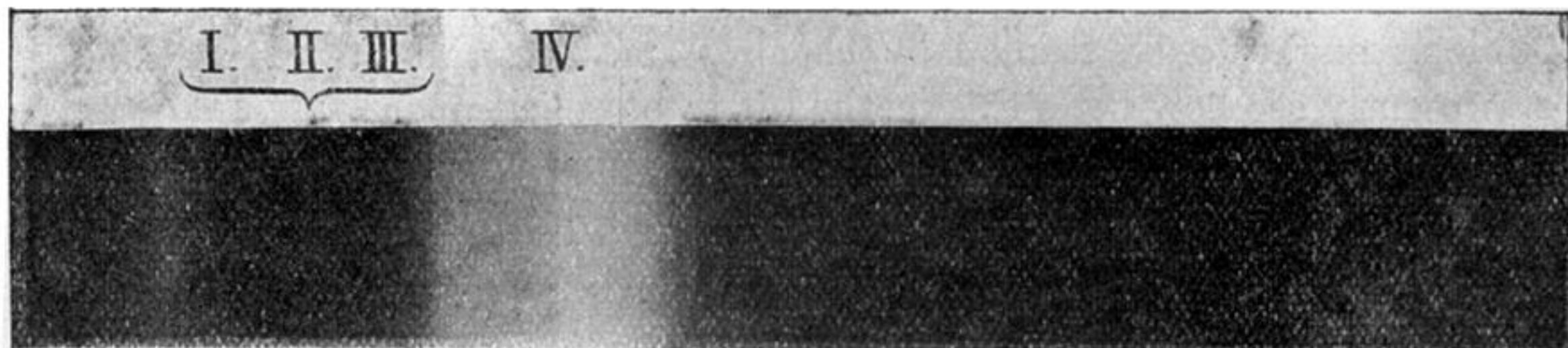
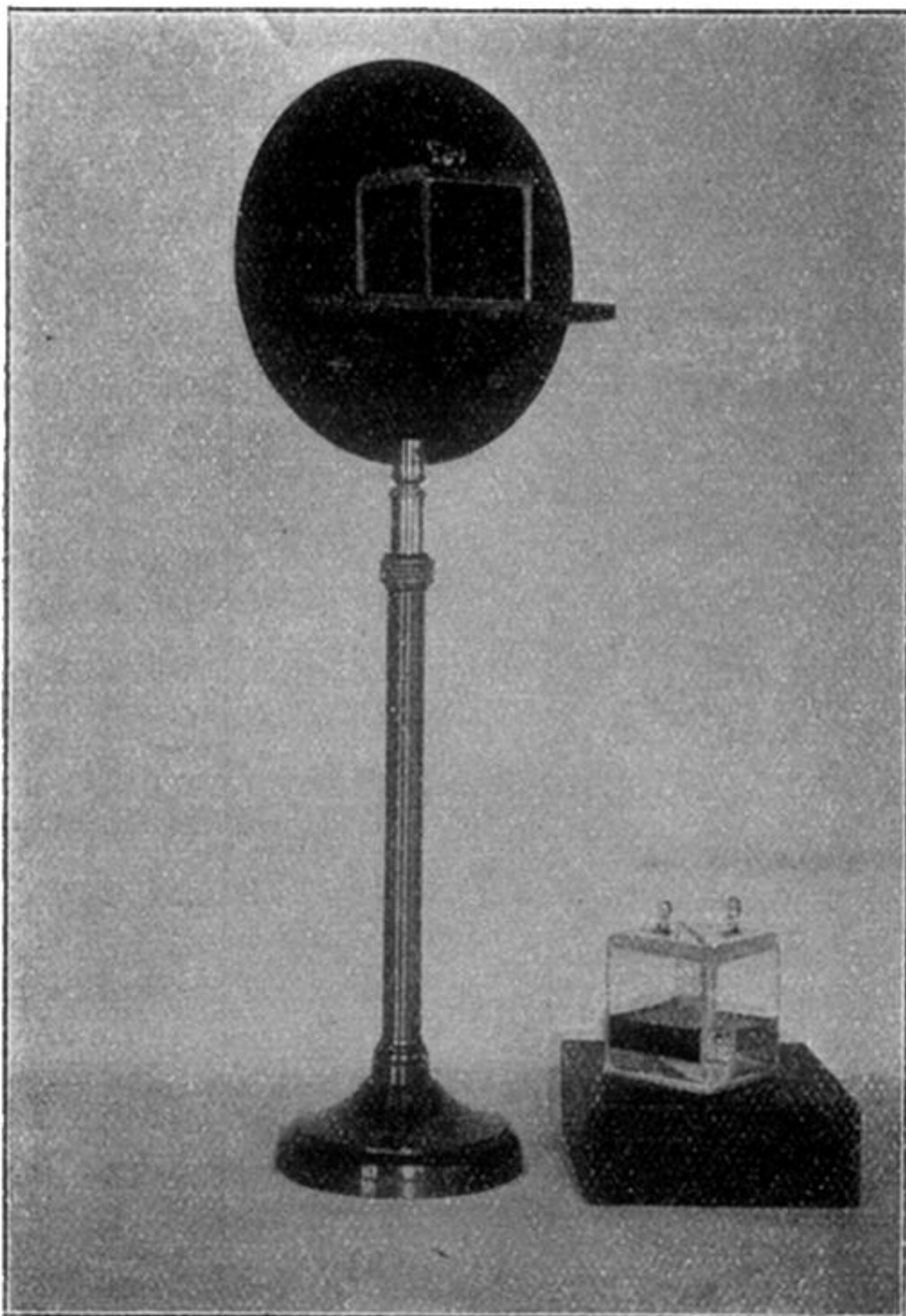Photographic Spectrum of Chlorophyll.
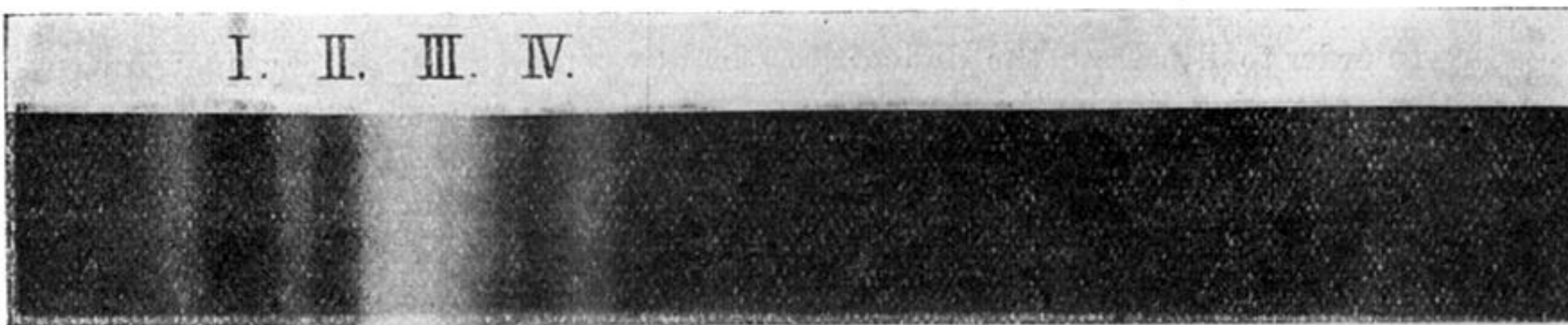
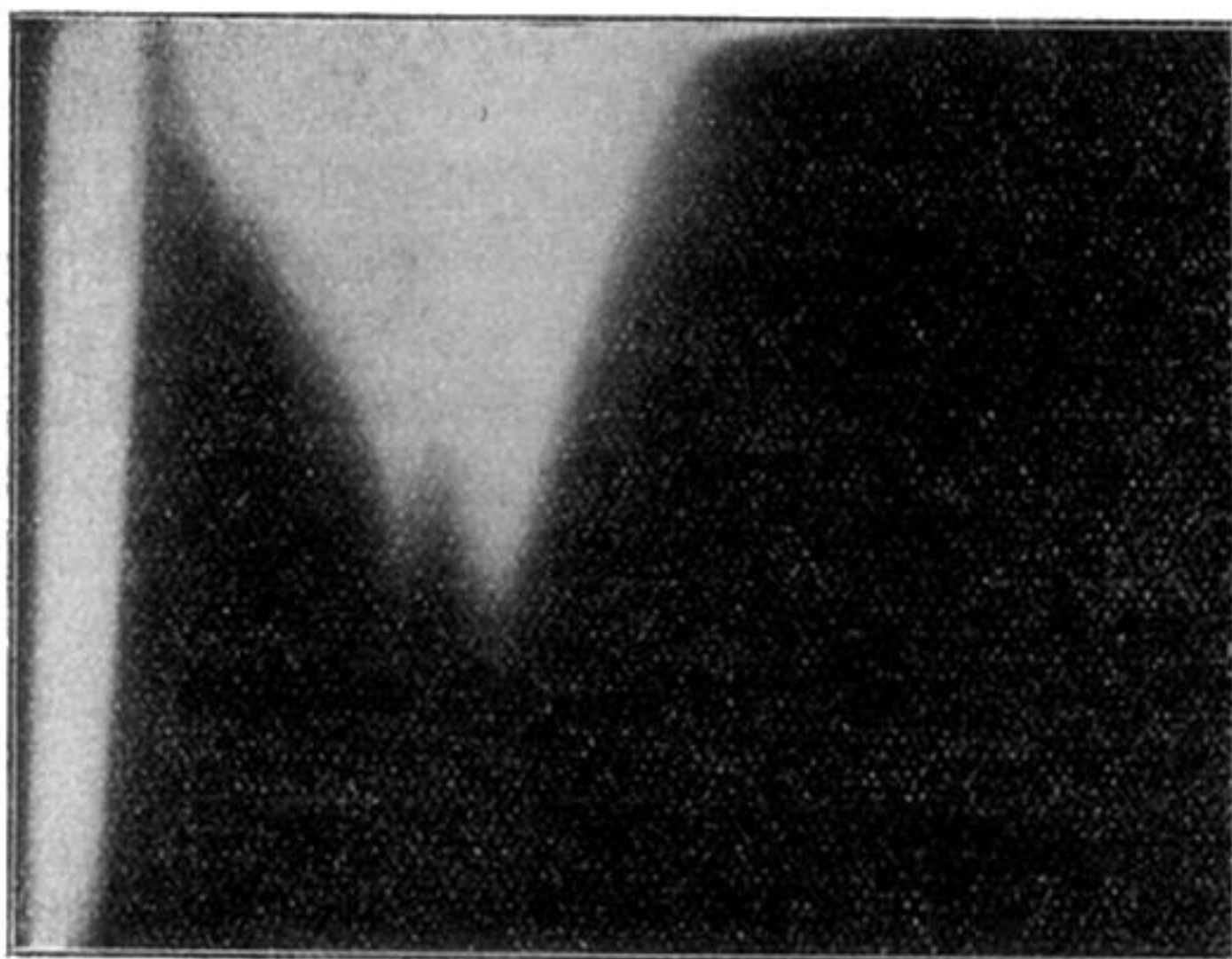Modified Chlorophyll (Stokes), same Concentration.

FIG. 3.

A.

B.

Apparatus for taking Photographs and the direct Projection of Spectrograms with the Lantern.
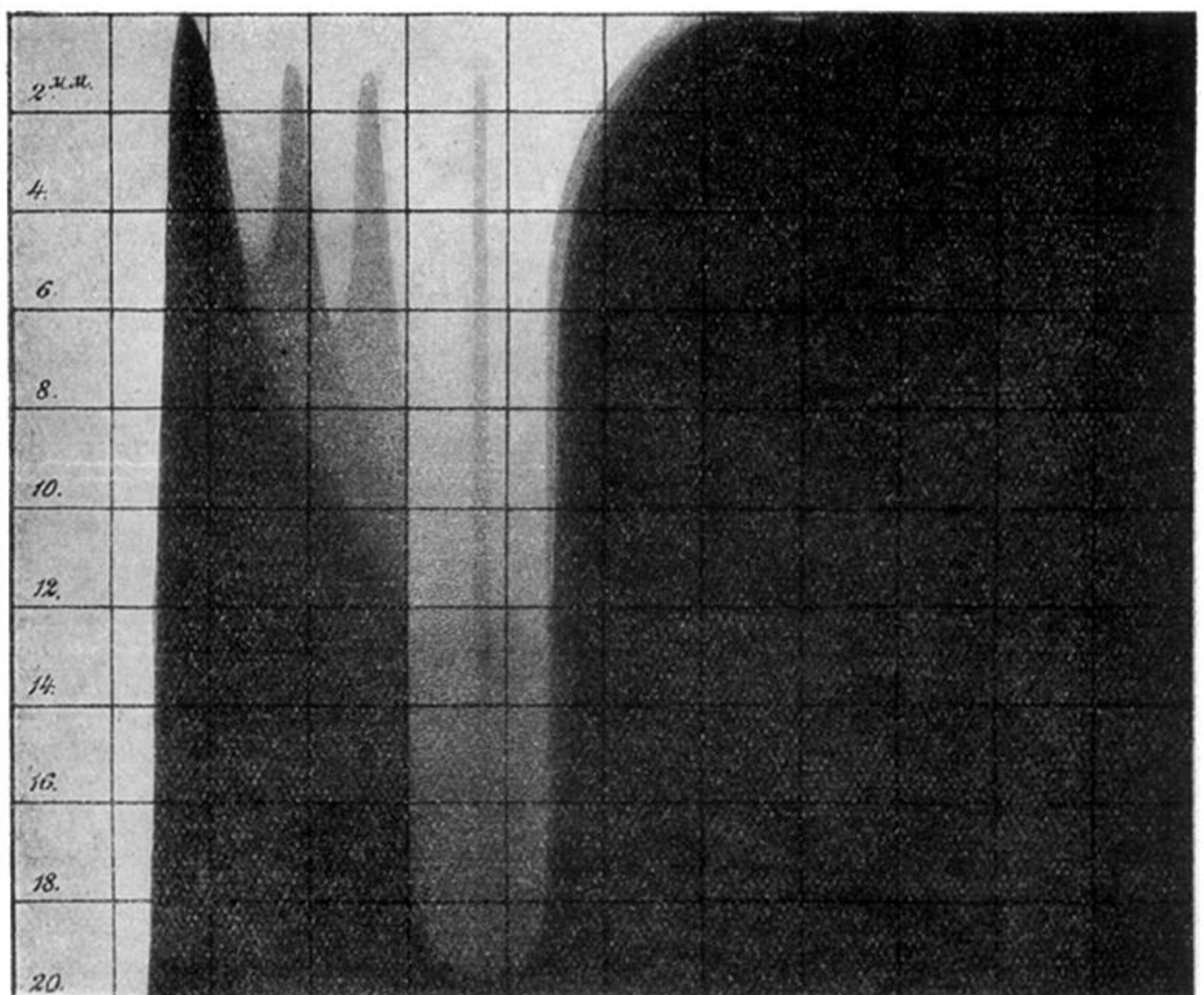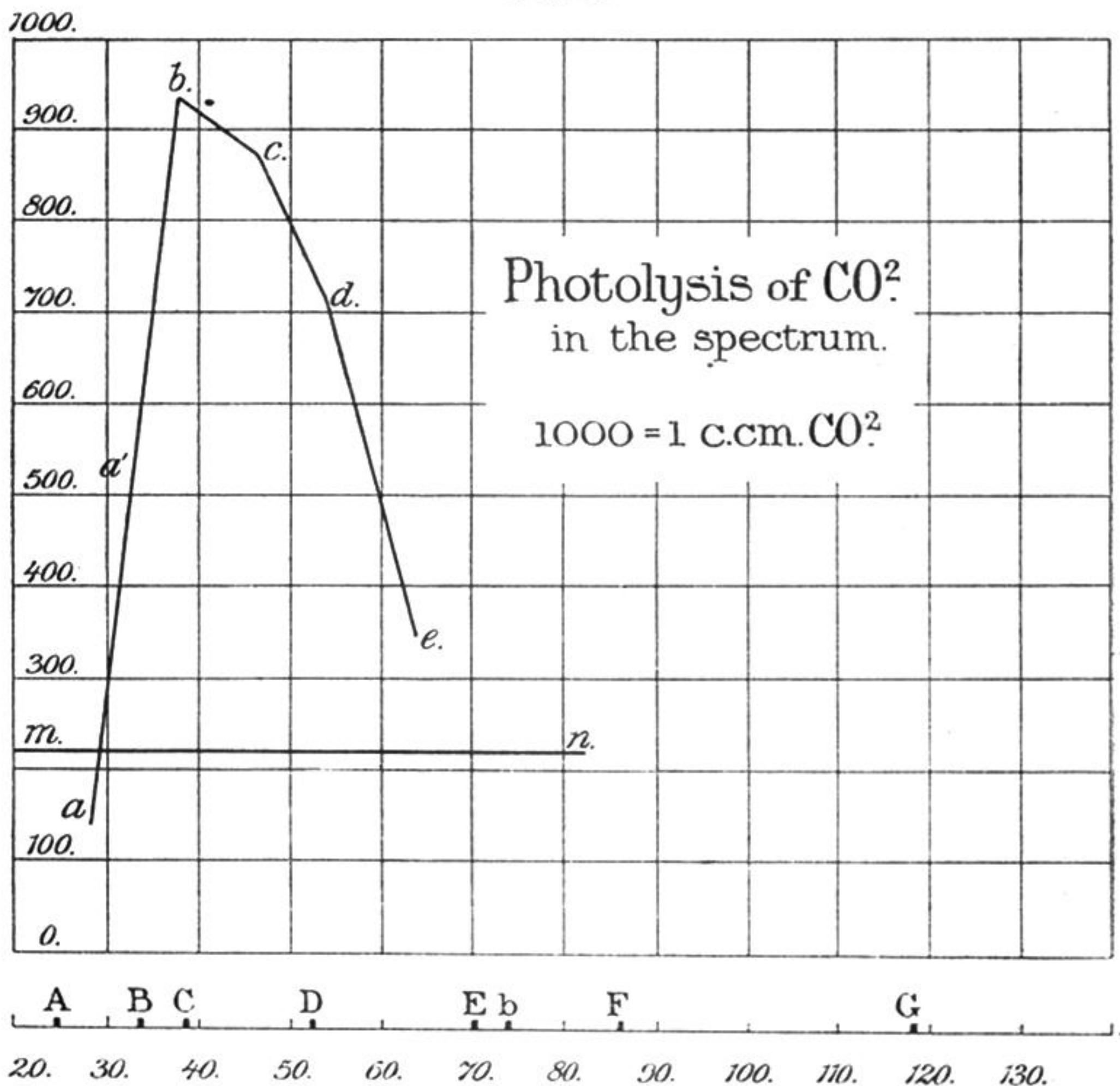
FIG. 4.

Photolysis of CO_2 in the spectrum.
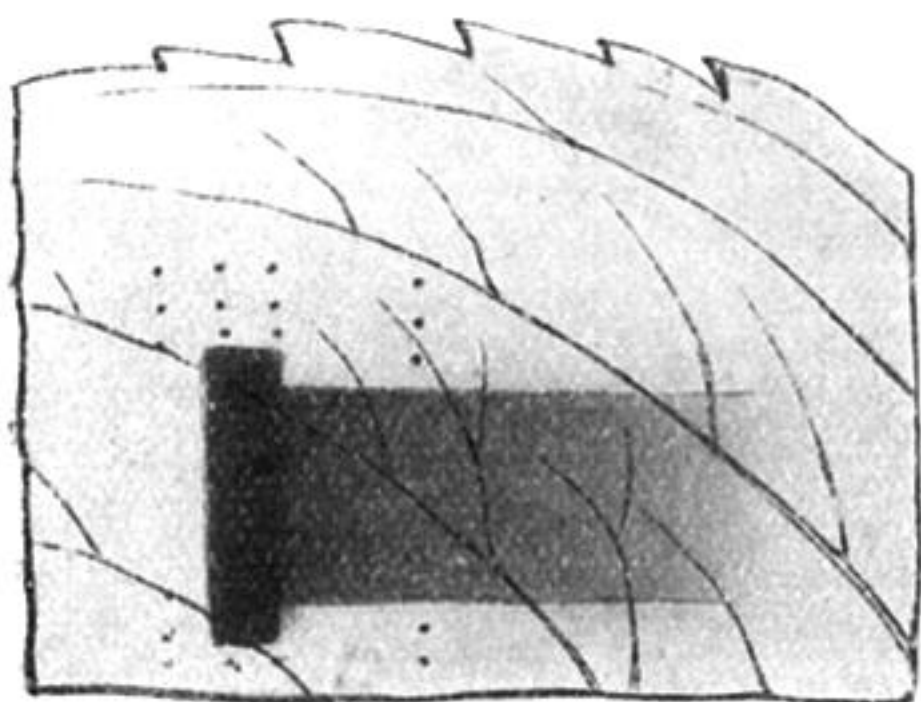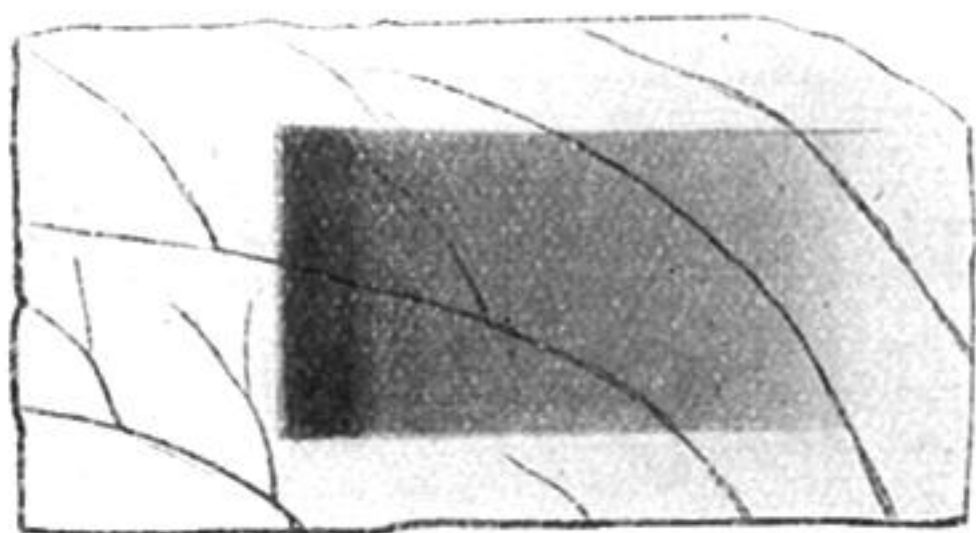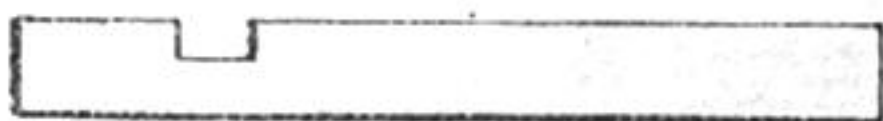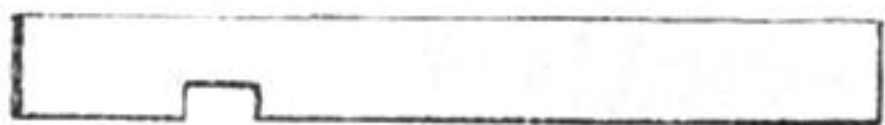
$1000 = 1$ c.cm. CO_2

FIG. 5.

a. B.C.   D.

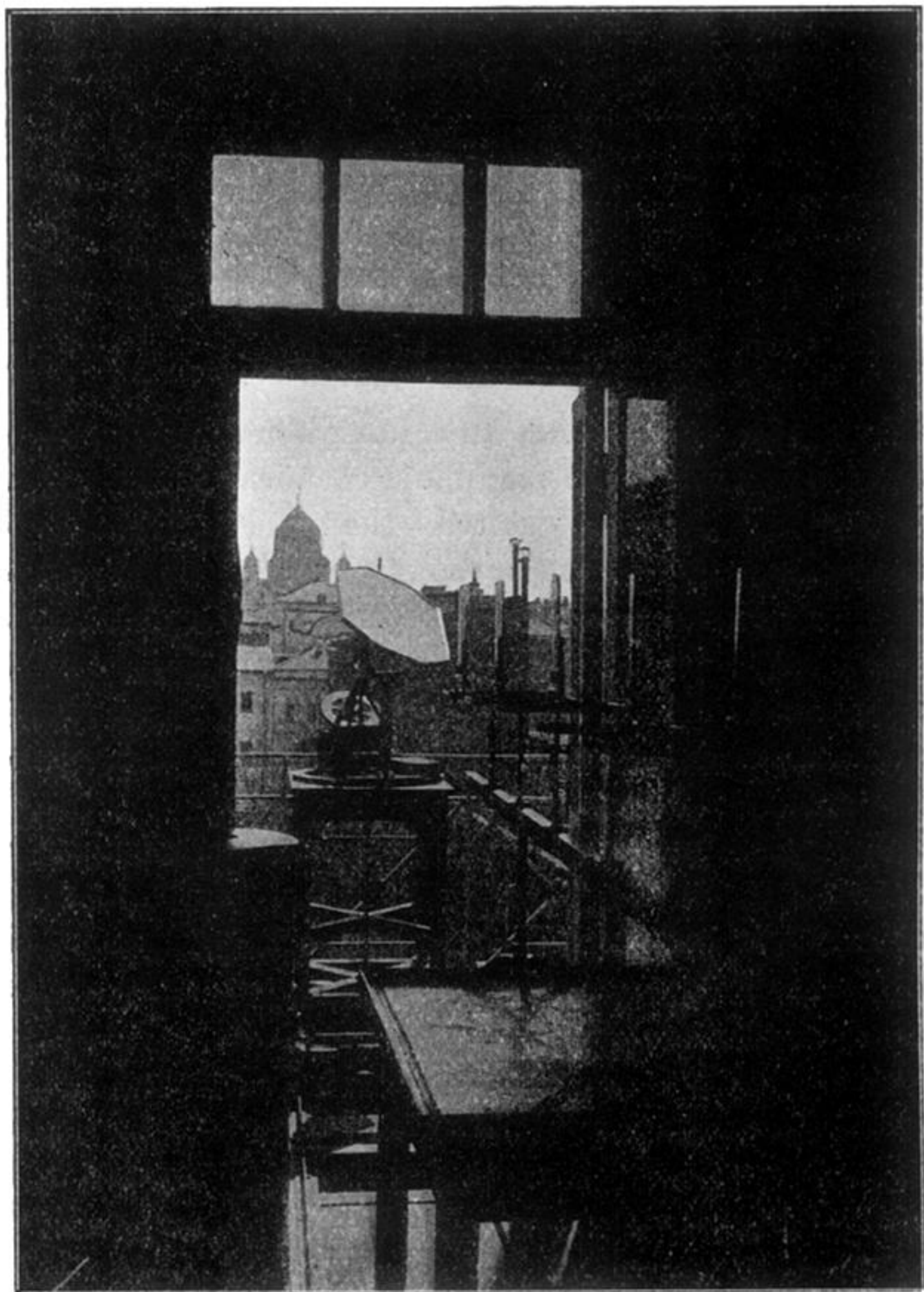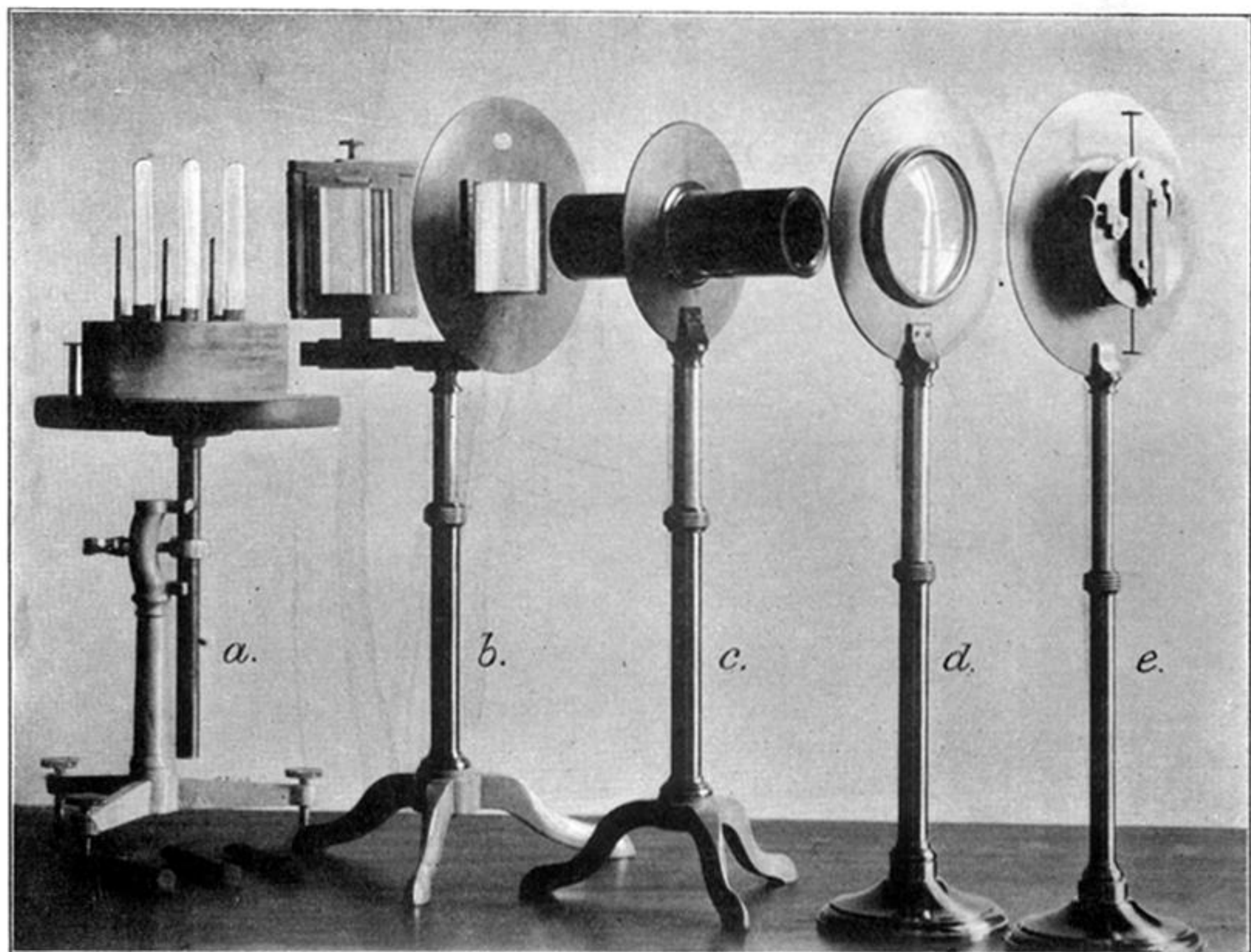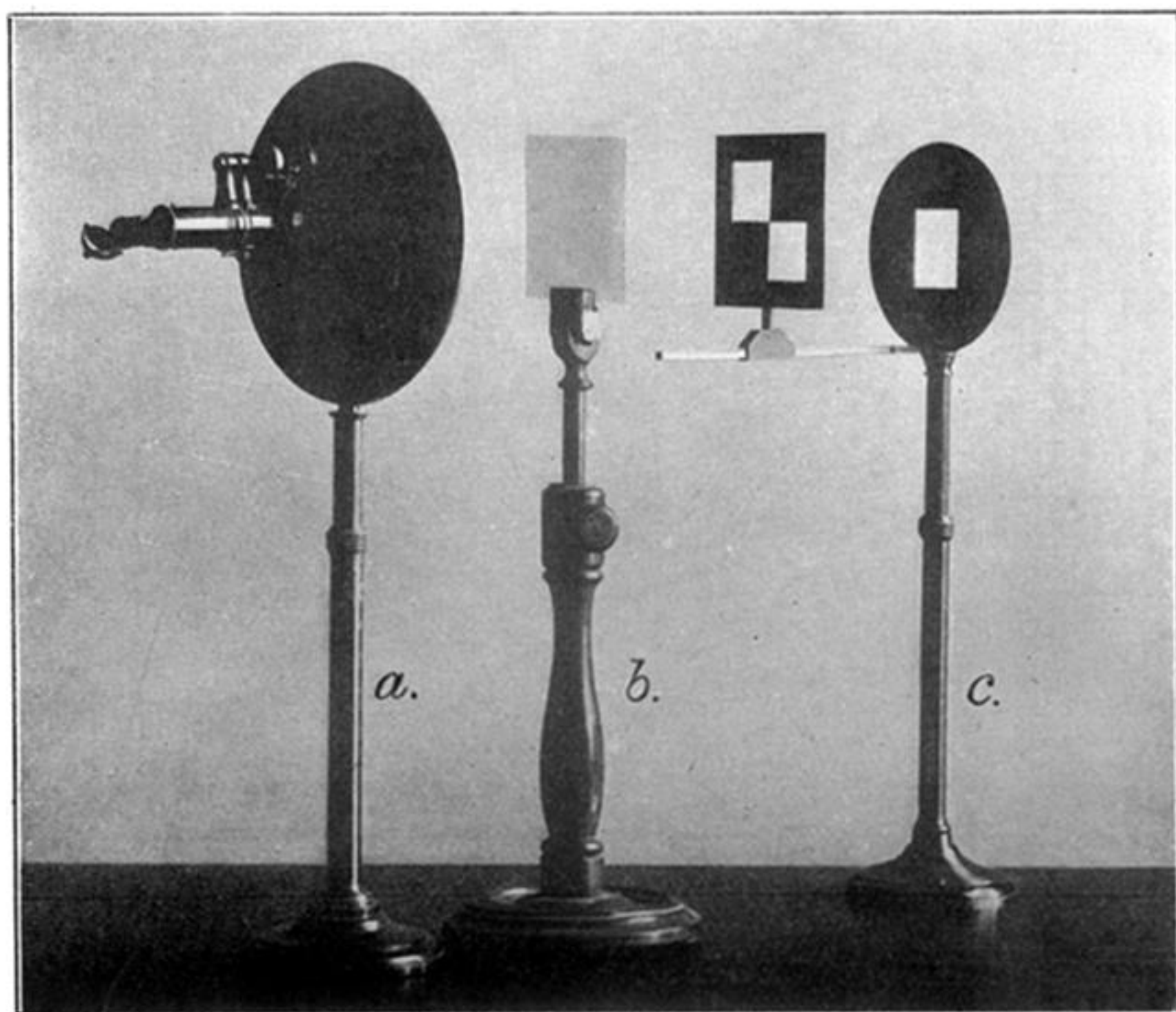Photosynthesis of $C_6H_{10}O_5$ in the Spectrum.

FIG. 7.

Yellow light.

Blue light.

Fig. 8.
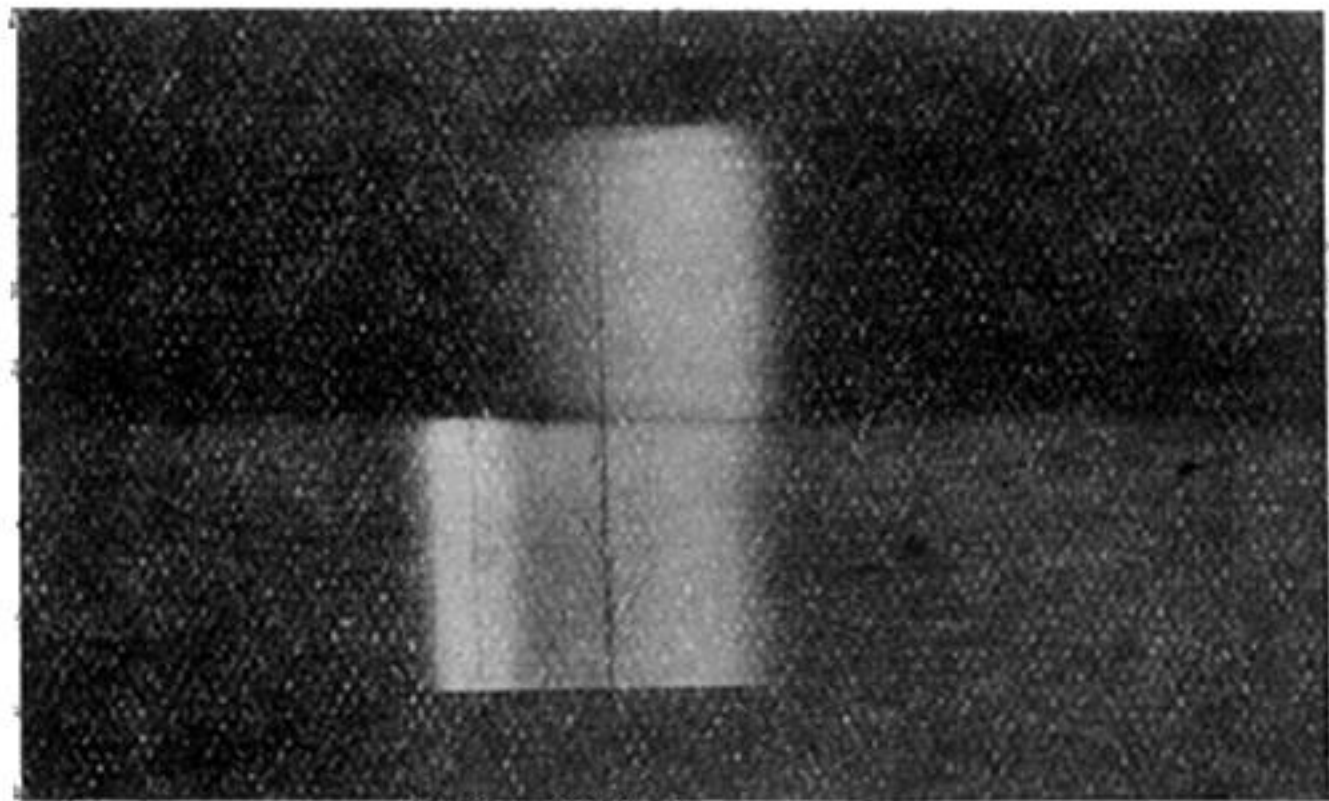
FIG. 9.

A

B

PLATE 20.

Apparatus for experimental research in coloured light of normal intensity (see page 436):

A. The spectrum is recomposed into two or three vertical bands lying side by side.

B. The spectrum is recomposed into two bands, superposed one over the other.
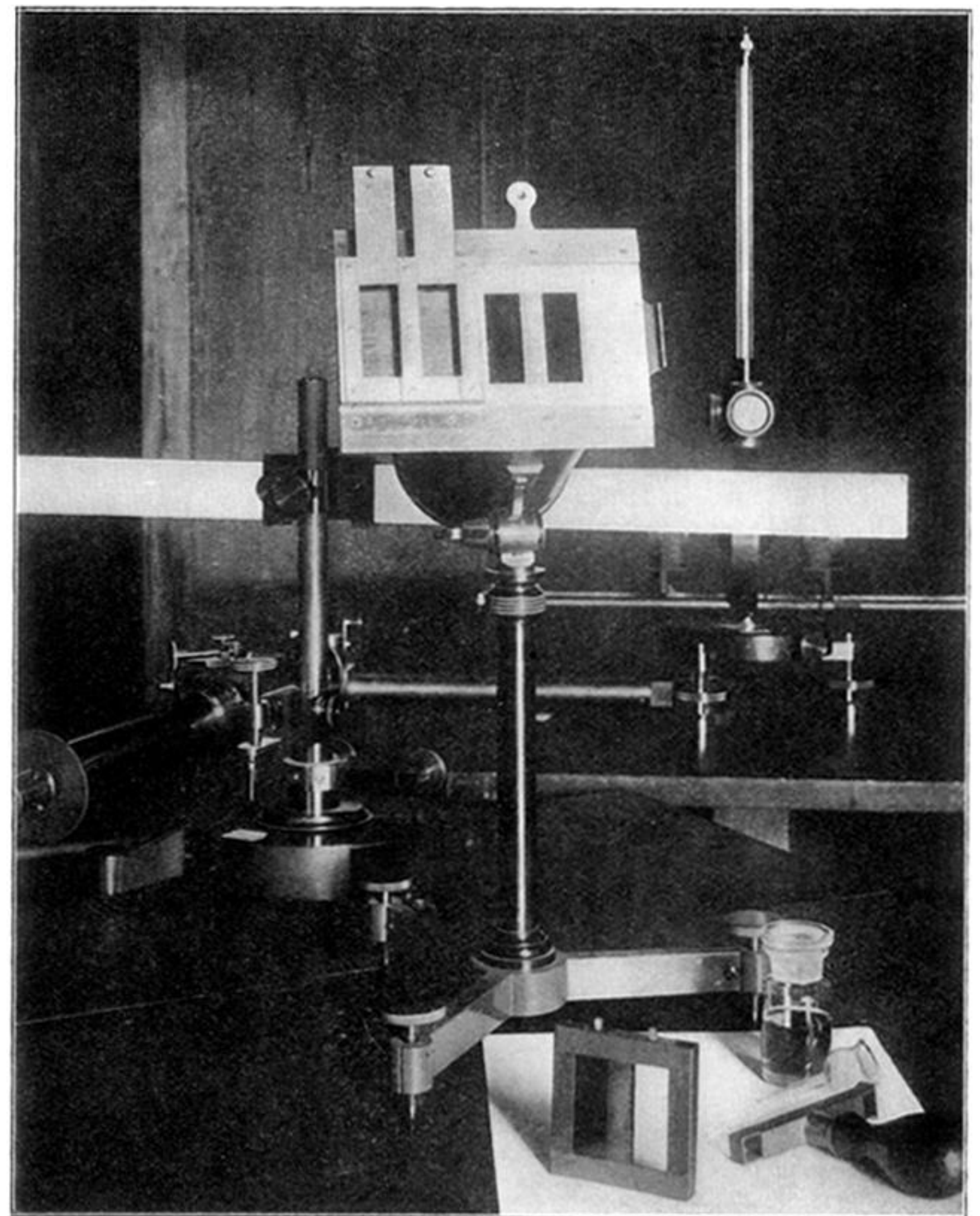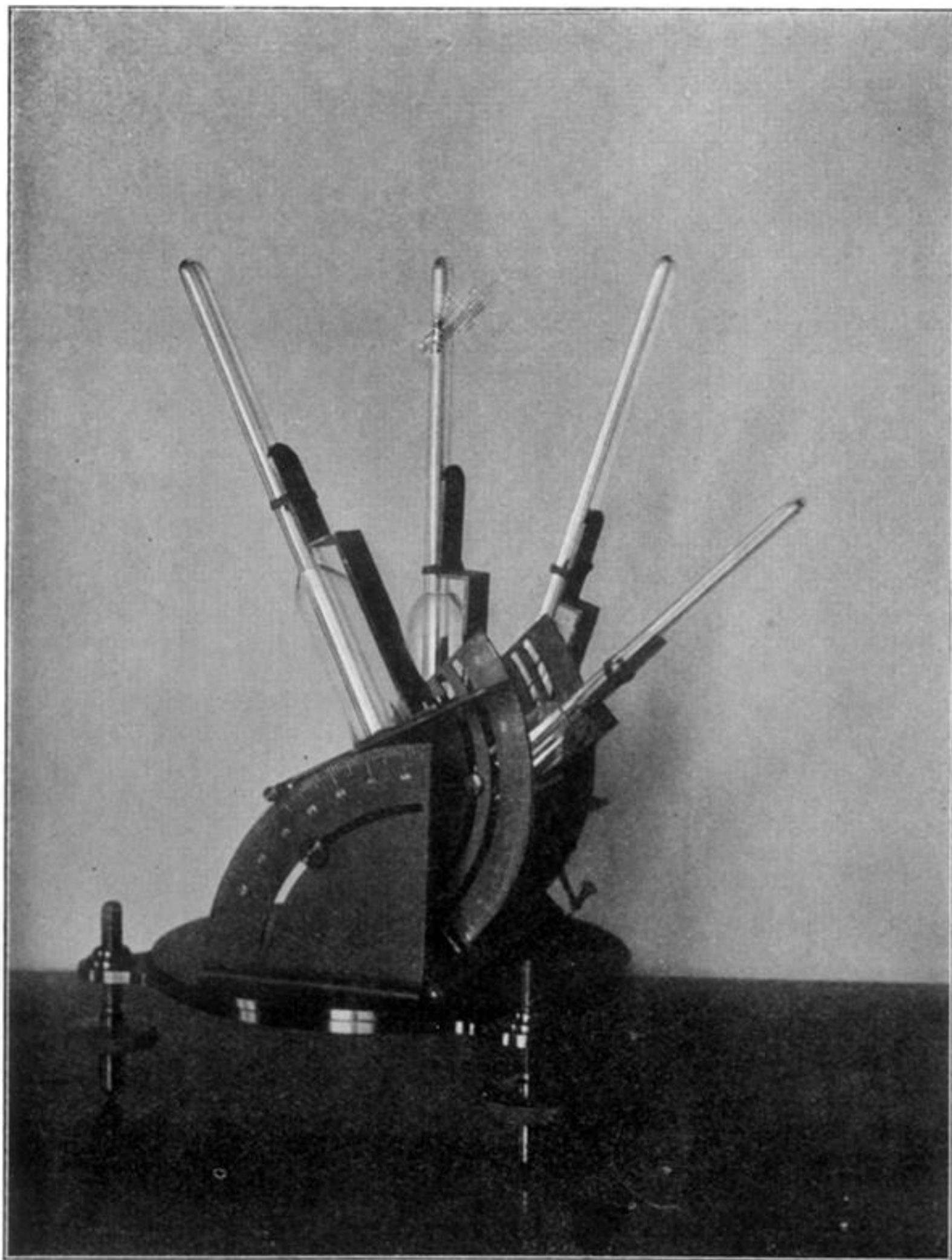
A

B

PLATE 21.

Phytoactinometer—apparatus for estimating the quantity of radiant energy
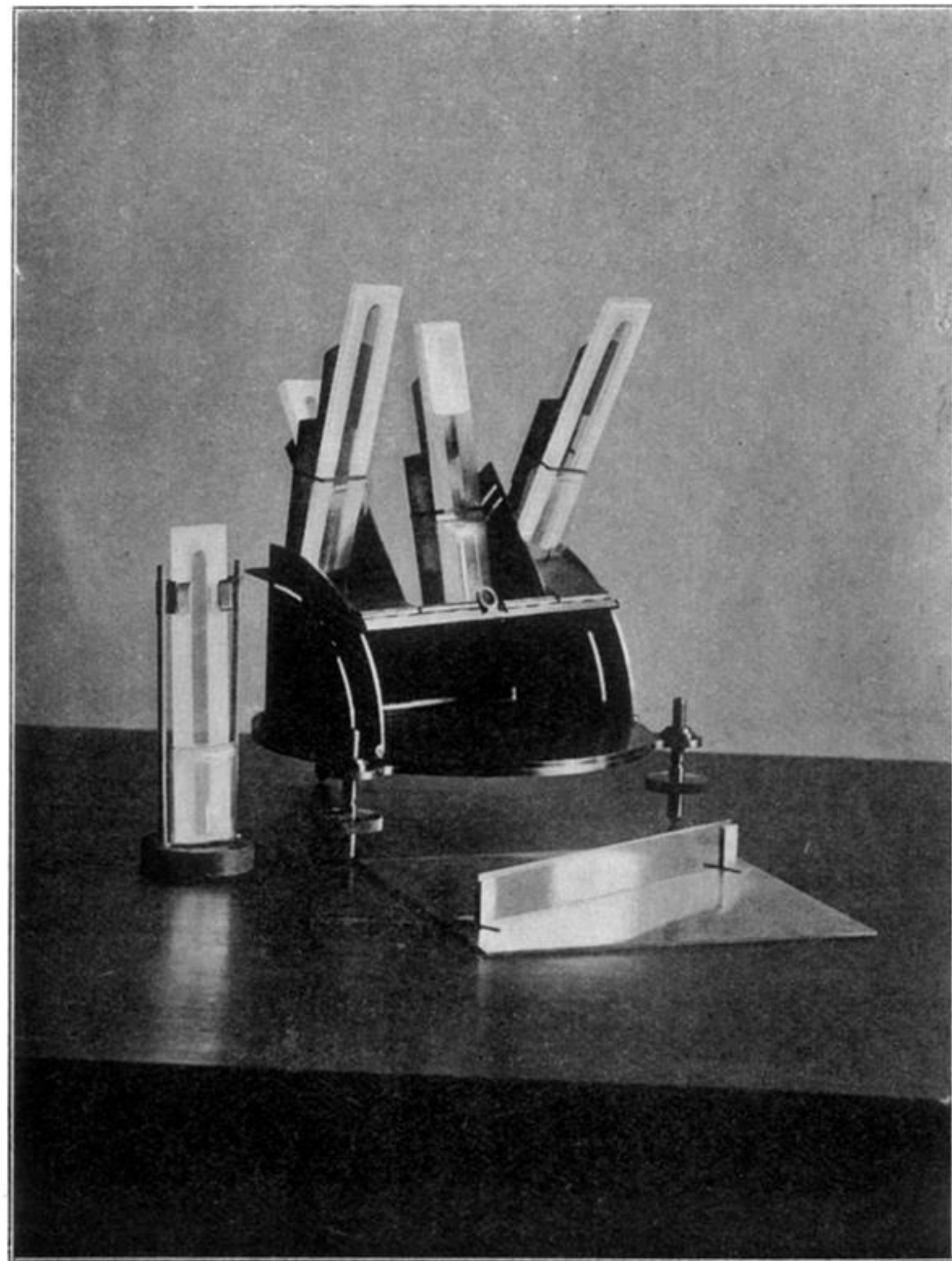absorbed by the chlorophyll of leaves (see p. 448).

PLATE 22.

Cosine photometer, for experimental research (see p. 450).